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Effects of Exposure to Heavy Metals on Selected Freshwater Fish. (Toxicity of Copper, Cadmium, Chromium & Lead to Eggs and Fry of Seven Fish Species.)

EG and G Bionomics, Wareham, Mass. Aquatic Toxicology Lab.

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Embryo and larvae of rainbow trout, lake trout, channel catfish, bluegill, white sucker, northern pike, and walleye were exposed for 60 days after hatch to lead and chromium in soft water. Brook trout, channel catfish, and walleyes were also exposed for 60 days after hatch to copper and cadmium in soft and hard water. The effects on survival and growth indicated that copper and cadmium were toxic at much lower concentrations than lead and chromium. Water hardness did not appear to have a significant effect on the observed toxicity in most cases.

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EFFECTS OF EXPOSURE TO HEAVY METALS ON SELECTED FRESHWATER FISH

Toxicity of Copper, Cadmium, Chromium and Lead to Eggs and Fry of Seven Fish Species

by

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FOREWORD

Our nation's freshwaters are vital for all animals and plants, yet our diverse uses of water---for recreation, food, energy, transportation, and industry---physically and chemically alter lakes, rivers, and streams. Such alterations threaten terrestrial organisms, as well as those living in water. The Environmental Research Laboratory in Duluth, Minnesota develops methods, conducts laboratory and field studies, and extrapolates research findings

--to determine how physical and chemical pollution affects aquatic life

--to assess the effects of ecosystems on pollutants

--to predict effects of pollutants on large lakes through use of models

--to measure bioaccumulation of pollutants in aquatic organisms that are consumed by other animals, including man

This report demonstrates the effects of lead, chromium, copper, and cadmium on the early life stages of rainbow trout, lake trout, brook trout, channel catfish, bluegill, white sucker, northern pike, and walleye. The results could be used in establishing water quality criteria for aquatic life.

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ABSTRACT

Eggs and fry of rainbow trout, lake trout, channel catfish, bluegill, white sucker, northern pike and walleye were continuously exposed for a maximum of 60 days after hatching, to a series of concentrations of lead and chromium in soft water (32.6-40.7 mg/l as $CaCO_3$). Similarly, eggs and fry of brook trout, channel catfish and walleyes were exposed to copper and cadmium in both soft (35.0-37.5 mg/l as $CaCO_3$) and hard (185.0-189.0 mg/l as $CaCO_3$) water. Observations of the hatchability of eggs and the survival and growth, quantitated as total length and wet weight, of fry were made after 30 and 60 days continuous exposure of the fry of these species to metals.

Results of exposures were used to estimate the range of metal concentrations which bracket the maximum acceptable toxicant concentration (MATC) for a given metal and fish species. The MATC's generated indicated that copper and cadmium were similar in toxicity and were toxic at significantly lower concentrations than lead and chromium. Water hardness did not appear to have a significant effect on the observed toxicity in most cases. Most importantly, MATC's estimated from these relatively short duration egg and fry tests were generally similar and in some cases, nearly identical to those estimated from chronic studies of much greater duration. This indicates that egg and fry studies are an effective, reliable means of assessing chronic toxicity of certain compounds to selected freshwater fish species in relatively short-term studies.

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SECTION I

INTRODUCTION

The establishment of a realistic and valid water quality criteria for chemicals entering surface waters requires an understanding of both the acute and chronic effects of these chemicals on aquatic organisms. Although the former is relatively easy to estimate, definition of the latter requires a time consuming and expensive effort. Data on estimates of acute toxicity of a myriad of chemicals to aquatic forms have been collected and are available (McKee and Wolf, 1963; Battelle-Columbus Laboratories, 1971). However, most of these data report the results of acute static bioassays and provide only a relative index of acute toxicity. Mount and Stephan (1967) and Brungs (1969) have described a method for estimating the maximum acceptable toxicant concentration (MATC) based on chronic toxicity studies. However, time consuming studies of this type have been performed for only a relatively small number of chemicals, primarily heavy metals and pesticides. Chronic studies have been completed using fathead minnows (*Pimephales promelas*) and copper (Mount, 1968; Mount and Stephan, 1969), zinc (Brungs, 1969) and cadmium (Pickering and Gast, 1972); with brook trout (*Salvelinus fontinalis*) and copper (McKim and Benoit, 1971, 1974); with rainbow trout (*Salmo gairdneri*) and lead (Davies and Everhart, 1973); and with brown bullheads (*Ictalurus nebulosus*) and copper (Christensen *et al*., 1972, Brungs *et al*., 1973). Similar studies have been performed with a number of pesticides (Mount and Stephan, 1967; Eaton,1970; Hermanutz *et al*., 1973; Macek *et al*., 1975, 1976, 1976).

These data suggest that the differences between acutely safe and chronically safe concentrations can be much greater for heavy metals than for pesticides. In addition, these data suggest that eggs and/or fry of fish may be the most sensitive life stages to chemical exposure. If this is a valid generalization, then it is possible that one could develop a realistic estimate of the MATC of a chemical for a fish species by defining the effects of that chemical on the eggs and fry. The successful development of such an approach would obviously result in a great saving of time and effort and/or reduce the need for the use of arbitrary application factors in the development of water quality criteria.

More recently, significant attention has been directed towards the evaluation of water chemistry (i.e., hardness, alkalinity and pH), and its influence on the toxicity of particular metal species to aquatic forms. Complexing properties of a series of chelating agents were evaluated on a number of heavy metals in order to determine mobilization and accumulation dynamics in biological tissue (Erickson, 1972). Laboratory studies with copper and fathead minnows, brook trout and rainbow trout indicated that this metal is highly complexed by carbonate and hydroxide ions as in natural waters and these phenomena determine the concentration of copper in solution. Further investigations suggested that alkalinity is the controlling factor in copper complexation (Pagenkopf _et al_., 1974). The ability of water hardness and pH to alter the toxicity of metals to fishes has also been investigated (Cairns and Scheier, 1957; Pickering and Henderson, 1965; Mount, 1966; Tabata, 1969).

The primary objective of this study was to empirically estimate the "no effect" levels of four heavy metals, lead, chromium, copper and cadmium, during continuous exposure of eggs and fry of eight species of fish in two types of water (40 and 200 mg/l hardness as $CaCO_3$). The fish species selected for this investigation were:

1. Rainbow trout (_Salmo gairdneri_)
2. Lake trout (_Salvelinus namaycush_)
3. Channel catfish (_Ictalurus punctatus_)
4. White sucker (_Catostomus commersoni_)
5. Bluegill (_Lepomis macrochirus_)
6. Northern pike (_Esox lucius_)
7. Walleye (_Stizostedion vitreum_)
8. Brook trout (_Salvelinus fontinalis_)

SECTION II

CONCLUSIONS

1. Cadmium and copper were similar in toxicity to the freshwater fish species tested and were significantly more toxic than were lead or chromium.

2. The sensitivity of all seven species of freshwater fish exposed to lead and chromium appears to be similar when MATC's are considered relative to the actual duration of exposure.

3. Fish eggs appeared to be the most resistant of the life cycle stages tested in this study. Hatchability of eggs was the parameter least affected by exposure to metals.

4. Water hardness did not appear to exert a significant influence on the effect of the metals tested on fry survival and growth over the long-term.

5. Scoliosis was an abnormality observed to have a significant incidence in fish exposed to lead.

6. The effects of both chromium and cadmium appeared to be cumulative during the entire exposure period.

7. Significant feeding problems experienced with northern pike and walleye may indicate that these species are unsuitable for this type of test.

8. Egg and fry tests appear to be effective and reliable short-term means of assessing potential hazards associated with environmental contaminants.

SECTION III

RECOMMENDATIONS

1. Egg and fry tests should be utilized for preliminary screening of all compounds whose potential hazard as environmental contaminants are unknown. If results of egg and fry tests are ambiguous then full-chronic assays should be performed.

2. The "Proposed Recommended Bioassay Procedure for Egg and Fry Stages of Freshwater Fish" should be revised on the basis of information generated in this study.

3. The recommended test temperature of 17° - 18°C for northern pike is too high. Temperatures of 10° - 12°C are more appropriate during egg incubation and hatching. During swim-up, exposure temperature should be increased gradually to 14° - 15°C.

4. Forage fish should be reared in large numbers concurrently with growing northern pike on a time scale to insure that forage fish size is 1/2 to 2/3 that of northern pike fry. A minimum of 5-10 appropriately-sized forage fish per northern pike fry is necessary to prevent cannibalism.

5. The recommended test temperature of 15°C for walleye is adequate for egg incubation and hatching but should be increased gradually to 18° - 21°C during swim-up to stimulate feeding.

6. Walleye fry test chambers should be entirely blackened on the sides, ends and bottom so that illumination is entirely from above. This results in more even distribution of fry in test tanks and improved feeding activity.

7. To improve survival and growth, fewer fry should be held in each growth chamber during chronic exposures. Reduction of the recommendation of 50 fry initially, thinned to 25 after 30 days should be reduced to 10-20 fry in each chamber during the entire 60 day exposure.

SECTION IV

MATERIALS AND METHODS

The methodology for these tests generally followed the Proposed Recommended Bioassay Procedure for Egg and Fry Stages of Freshwater Fish, issued by the National Water Quality Laboratory, Duluth, Minnesota (U.S. EPA, 1972 a).

EXPOSURE SYSTEMS

Proportional diluters (Mount and Brungs, 1967) with a dilution factor of 0.5 were utilized for the delivery of six toxicant concentrations and a control to two replicate test aquaria. Chambers to promote mixing of toxicant and diluent water were utilized prior to the delivery of water to the test aquaria which measured 35 x 30 x 30 cm with a water depth of 20 cm. Each test chamber was divided to provide space for two growth chambers which measured 25 x 30 x 12.5 cm and had stainless steel screening (40 mesh) attached to one end to allow water to drain to a depth of 2.5 cm upon removal from the aquaria. This design allowed for the transfer of growth chambers to a fluorescent light box for photographing the fish. Test aquaria were maintained in circulating water baths at the recommended temperature for the species being tested. Egg incubation cups, made from 4-oz., 2-in. OD round glass jars with the bottoms cut off and replaced with nylon screen (40 mesh), were oscillated in the test water by means of a rocker arm apparatus driven by a 2 rpm motor (Mount, 1968). Eggs and fry were shielded from all sources of light by means of black polyethylene curtains.

Diluent water obtained from a 400 ft. bedrock well had a measured total hardness of 35 mg/l (as $CaCO_3$). Well water was delivered to test units through PVC pipes in the soft water exposures. In the hard water exposures, well water was passed through a water hardner as described by Lemke (1969). The hardner utilized the principle of carbonate/bicarbonate ion buffering system and supplied water with a nominal hardness of 180 mg/l (as $CaCO_3$).

CHEMICAL METHODS

Water quality parameters such as acidity, alkalinity, hardness and pH of diluent water were measured according to the methods described in APHA *et al*. (1971). Dissolved oxygen concentration of test water was measured with a YSI Oxygen Meter and oxygen/temperature probe.

Stock solutions of the appropriate metal salts were dissolved in distilled water and delivered to diluters from a Mariotte bottle via a volumetric delivery system. Analytical grade chemicals utilized to obtain respective metal stock solutions were cadmium chloride ($CdCl_2$), sodium dichromate (Na_2CrO_7), copper sulfate ($CuSO_4 . 5H_2O$) and lead nitrate [$Pb(NO_3)_2$].

The analyses of metals in hard and soft water were performed by atomic absorption spectroscopy using conventional acetylene-air flame aspiration of the sample. In the case of several of the lower concentrations of lead, copper and cadmium in water samples, residues were concentrated by solvent extraction prior to flame atomization and analysis.

Distilled water was obtained from a Corning AG-1 all-glass still and passed through a Barnstead mixed-bed ion exchange cartridge before use. An analytical standard of each of the four metals was prepared separately in deionized distilled water at a concentration of 1,000 mg/l. These four standard solutions were used throughout the study, however, dilutions of the analytical standards were made at the time of analysis and discarded after use. Metal standards were: lead nitrate, #2322, Lot 45008, J.T. Baker; chromium trioxide, #A-99, Lot 731023, Fisher Scientific; copper metal, #1736, Lot 43671, J.T. Baker; and cadmium metal, #CX13-CB1-52, Lot 16, Matheson, Coleman and Bell.

Organometallic compounds used to standardize instrument response prior to analyzing water extracts were: lead cyclohexanebutyrate, #10395, Eastman; copper cyclohexanebutyrate, #10389, Eastman; and cyclohexanebutyric acid, cadmium salt, #10386, Eastman. No standardization of this type was necessary for chromium as it was always measured directly and never required solvent extraction.

Lead, chromium and copper standards were prepared and diluted in deionized distilled water according to U.S. EPA (1971). The cadmium standard was prepared similarly according to Perkin-Elmer (1971).

Organometallic compounds were dried for 48 hours in a desiccator over fresh phosphorus pentoxide, then weighed and diluted with methyl isobutyl ketone (MIBK) (#453, Burdick & Jackson) to concentrations of 100 mg of metal per liter of MIBK. Further dilutions of all metal standard solutions used to calibrate instrument response, were prepared on the day of analysis and discarded after use.

All analyses of metals were performed with a Perkin-Elmer Model 305-A atomic absorption spectrophotometer, using an acetylene-air flame. The instrument contains a factory-installed deuterium background corrector which was used in these analyses. Instrument response to light absorption by the metal of interest was measured with a Perkin-Elmer Model 56 strip chart recorder.

Water samples (unfiltered) were collected in high-density polyethylene bottles which were cleansed and prepared for use according to U.S. EPA (1972 b).

Concentrated nitric acid was added to each water sample (one percent by volume) and the sample was stored at room temperature prior to analysis (2-3 weeks). As a check for contamination, a solution of one percent nitric acid in deionized distilled water was analyzed for lead, chromium, copper, and cadmium. No detectable concentrations of these metals were present in the solution. No suppression or enhancement of signal response was observed when known concentrations of these metals were added to the solution and the signal response obtained was compared to the response of analytical standards containing from 0.1 to 5 percent nitric acid.

Concentrations of lead in water to 60 µg/l, copper to 25 µg/l and cadmium to 25 µg/l were analyzed by direct sample atomization. Metal concentrations less than those stated above were measured by extracting the metal solution, according to U.S. EPA (1971), and aspirating the MIBK extract. The percentage metal recovery of the extraction procedure was accounted for in the procedure since the absorbance of samples was compared to the absorbance of inorganic metal standards which were also extracted in the same manner.

The extraction procedure concentrates the metal by a factor of ten in the MIBK solvent. In addition, the absorbance of lead, copper and cadmium in MIBK solvent was ca four times the absorbance of these metals in aqueous solution. Therefore, the effective concentration achieved by extracting the metal was 40X.

All concentrations of chromium were determined by direct atomization of the sample.

AA operating conditions; acetylene-air flame with three-slot burner atomizing 6 ml of water or 4 ml of MIBK per minute.

Lead: Analytical wavelength - 2170 Angstroms
Bandpass - 0.7 Angstroms
Recorder - 0.060 to 0.200 mg/l range; 27 and 87 mm peak heights, respectively.

Chromium: Analytical wavelength - 3579 Angstroms
Response - a. 0.015 to 0.500 mg/l range; 6 and 212 mm peak heights, respectively.
Response - b. 0.015 to 0.200 mg/l range: 14 and 176 mm peak heights, respectively.

All other conditions were the same as for "lead" above.

Copper: Analytical wavelength - 2347 Angstroms
Response - 0.025 to 0.100 mg/l range; 40 and 151 mm peak heights, respectively.

All other conditions were the same as for "lead" above.

Cadmium: Analytical wavelength - 2288 Angstroms
Response - 0.025 to 0.200 mg/l range; 30 and 225 mm peak heights, respectively.

All other conditions were the same as for "lead" above.

Standard deviation of the measurement: Measurement involves placing a nebulizer tube into the sample or standard solution, allowing 20-90 seconds for the recorder pen response to stablize and then returning the nebulizer tube to deionized distilled water to obtain a baseline reading. The pen response to standard solutions was the same when aspiration occurred continuously for two minutes or for five separate trials, therefore the standard deviation is essentially the error involved in measuring the peak heights with a millimeter ruler (i.e., very small).

BIOLOGICAL METHODS

Egg exposures were initiated by randomly selecting two groups of 100 eggs each for each replicate tank, i.e. 400 eggs per concentration. Egg mortality was recorded daily and dead eggs were removed to prevent fungus growth. When hatching commenced, the number of eggs hatching in each group was recorded daily until hatching was completed. Surviving fry were impartially reduced to two groups of 50 per replicate tank, (200 fry per concentration) and placed in the duplicate growth chambers. Survival was recorded daily and after 30 days, fish were transferred to a box with a translucent millimeter grid for photographic determination of total (tip of snout to end of caudal fin) length (McKim and Benoit, 1971). At this time, the remaining fry were impartially reduced to 25 per growth chamber (100 per concentration) and returned to the appropriate test chambers. After 60 days post-hatch, photographs for growth measurements were repeated, total group wet weights were determined and the experiment was terminated.

Specific information on source of test organisms, incubation data and feeding for each species is presented in Table 1. All fry were fed a minimum of four times daily. All aquaria were siphoned daily to remove fecal material, excess food and detritus and aquaria were brushed when algal growth became excessive.

STATISTICS

Measured biological parameters from duplicate containers during continuous exposure were averaged and subjected to analysis of variance according to Steele and Torrie (1960). When treatment effects were indicated, the means of these effects were subjected to Duncan's Multiple Range Test to determine which treatments were statistically different from the controls. All differences were considered significant at a probability of 0.05.

TABLE 1 - SOURCE, AGE OF EGGS AT BEGINNING OF EXPOSURE, INCUBATION TIME AND TEMPERATURE, AND FOOD UTILIZED IN THE EXPCSURE OF EGGS AND FRY OF SELECTED FRESHWATER FISH SPECIES TO METALS

Species	Source	Age	Incubation time	Temp. (°C) $\bar{x}$ ± S.D.	Food
Rainbow trout (*Salmo gairdneri*)	Wareham, Mass.	0-1 day	35-37 days	10 ± 1	Oregon moist trout starter
Lake trout (*Salvelinus namaycush*)	Laconia, N.H.	0-1 day	51-55 days	10 ± 1	Oregon moist trout starter
Channel catfish (*Ictalurus punctatus*)	Lonoke, Ark.	2-3 days	6-8 days	22 ± 1	ground liver, trout starter
White sucker (*Catostomus commersoni*)	Laconia, N.H.	0-1 day	10-13 days	17 ± 1	daphnids, brine shrimp nauplii, trout starter
Bluegill (*Lepomis macrochirus*)	Wareham, Mass.	<12 hours	2 days	25 ± 1	mixed zooplankton, daphnids, brine shrimp nauplii
Northern pike (*Esox lucius*)	Lansing, Iowa	5 days (eyed)	4 days	17 ± 1	daphnids, white sucker fry
Walleye (*Stizostedion vitreum*)	Spirit Lake, Iowa	2 days	9-12 days	15 ± 1	brine shrimp nauplii, daphnids
Brook trout (*Salvelinus fontinalis*)	Wareham, Mass.	0-1 day	35 days	10 ± 1	Oregon moist trout starter

SECTION V

RESULTS

WATER CHEMISTRY

Results of the chemical analysis of water samples indicated that chemical characteristics were not significantly variable among treatments within a test. Therefore, only means (and standard deviations) and ranges for the various parameters in each test are presented (Tables 2, 3, 4, 5).

The results of the atomic absorption analysis of water samples taken periodically during continuous exposure to materials indicated that mean measured concentrations closely approximated nominal concentrations and were relatively constant throughout exposure (Tables 6, 7, 8, 9). The notable exceptions to this were the two instances where we attempted to continuously expose rainbow trout and lake trout to 1000 μg/l dissolved lead. In both cases we were able to measure only 480-672 μg/l with the remainder occurring as a precipitate at the bottom of the aquaria.

EXPOSURE OF FISH TO LEAD

Rainbow trout

The percentage of rainbow trout eggs successfully hatching when continuously exposed to 672 μg/l lead during incubation was significantly lower than the percentage hatch observed among control eggs and those exposed to lesser concentrations of lead (Table 10). Survival of trout fry continuously exposed to 672, 443 and 250 μg/l lead for 30 days was significantly lower than fry survival in the other three concentrations and the controls. Further evidence of the effect of exposure to lead concentrations of 250 μg/l and higher on trout fry was the significantly smaller total lengths of survivors in these concentrations after 30 days continuous exposure when compared with controls.

After 60 days exposure to 250 μg/l lead only two juvenile rainbow trout had survived and no fish survived 60 days continuous exposure to 443 and 672 μg/l. Survival among rainbow trout continuously exposed to 146 μg/l lead was slightly but not significantly reduced after 30 days. However, survival had decreased significantly during the 30-60 day exposure interval indicating a cumulative effect of lead at this concentration.

Total lengths and wet weights of rainbow trout at the termination of exposure (60 days post-hatch) were similar for controls and all lead concentrations with surviving fish.

TABLE 2 - MEANS AND STANDARD DEVIATIONS ($\bar{x}$ ± S.D.), RANGES AND NUMBER OF OBSERVATIONS (N) OF MEASURED WATER QUALITY PARAMETERS DURING EXPOSURE OF EGGS AND FRY OF SELECTED FRESHWATER FISH SPECIES TO LEAD

	Parameter				
Species	Acidity (mg/l)	Alkalinity (mg/l as $CaCO_3$)	Hardness (mg/l as $CaCO_3$)	Dissolved O_2 (mg/l)	pH
Rainbow trout					
$\bar{x}$ ± S.D.	3.5±1.1	30.2±3.8	34.6±3.2	10.3±0.9	-
Range	2.9-4.8	24.0-33.0	32.0-42.0	8.6-11.3	6.9-7.4
(N)	(14)	(14)	(14)	(118)	(14)
Lake trout					
$\bar{x}$ ± S.D.	3.4±1.1	29.6±4.1	32.6±3.9	9.7±1.4	-
Range	1.9-4.0	23.1-34.1	25.0-38.0	8.6-11.3	7.0-7.3
(N)	(16)	(16)	(16)	(182)	(16)
Channel catfish					
$\bar{x}$ ± S.D.	3.4±0.8	33.6±1.3	36.0±1.0	8.5±0.7	-
Range	2.9-4.8	31.9-35.2	24.5-37.0	6.5-9.2	6.8-7.3
(N)	(6)	(6)	(6)	(99)	(6)
White sucker					
$\bar{x}$ ± S.D.	3.5±0.9	34.8±4.5	37.6±5.9	8.8±0.6	-
Range	1.9-4.8	31.9-42.0	32.0-48.0	7.3-9.8	6.7-7.1
(N)	(8)	(8)	(8)	(96)	(8)
Bluegill					
$\bar{x}$ ± S.D.	5.3±0.9	33.3±3.8	40.7±7.3	6.9±2.0	-
Range	4.0-6.2	28.0-36.0	35.0-53.0	5.0-8.7	6.7-7.2
(N)	(6)	(6)	(6)	(203)	(8)
Northern pike					
$\bar{x}$ ± S.D.	3.6±1.2	33.1±3.8	35.4±4.8	8.7±0.6	-
Range	2.9-6.0	30.0-42.0	31.0-45.0	7.6-9.3	6.7-7.3
(N)	(8)	(8)	(8)	(84)	(8)
Walleye					
$\bar{x}$ ± S.D.	3.7±1.0	33.8±4.0	37.6±5.2	10.3±1.1	-
Range	2.9-4.8	31.9-42.0	32.0-45.0	9.2-10.4	6.7-7.1
(N)	(4)	(4)	(4)	(68)	(4)

TABLE 3 - MEANS AND STANDARD DEVIATIONS ($\bar{x}$ ± S.D.), RANGES AND NUMBER OF OBSERVATIONS (N) OF MEASURED WATER QUALITY PARAMETERS DURING EXPOSURE OF EGGS AND FRY OF SELECTED FRESHWATER FISH SPECIES TO CHROMIUM

	Parameter				
Species	Acidity (mg/l)	Alkalinity (mg/l as $CaCO_3$)	Hardness (mg/l as $CaCO_3$)	Dissolved O_2 (mg/l)	pH
Rainbow trout					
$\bar{x}$ ± S.D.	3.3±1.2	30.1±4.0	33.4±4.5	9.1±0.6	-
Range	1.0-4.8	23.0-34.1	30.0-42.0	6.7-12.2	6.7-7.0
(N)	(16)	(16)	(16)	(288)	(14)
Lake trout					
$\bar{x}$ ± S.D.	3.6±1.1	31.5±2.8	34.0±4.8	9.5±0.9	-
Range	1.9-4.8	24.2-33.0	31.0-42.0	6.7-12.2	6.8-7.1
(N)	(14)	(14)	(14)	(340)	(14)
Channel catfish					
$\bar{x}$ ± S.D.	4.0±0.7	33.7±1.3	36.2±1.2	8.1±1.5	-
Range	2.9-4.8	31.9-35.2	35.0-38.0	5.6-9.4	7.0-7.4
(N)	(6)	(6)	(6)	(87)	(6)
White sucker					
$\bar{x}$ ± S.D.	3.2±0.7	34.6±5.6	38.8±5.3	8.9±0.6	-
Range	2.9-4.0	30.0-44.0	32.0-46.0	7.5-10.4	6.9-7.2
(N)	(8)	(8)	(8)	(96)	(8)
Bluegill					
$\bar{x}$ ± S.D.	6.6±1.1	33.0±5.3	38.3±4.6	6.6±1.3	-
Range	5.0-7.8	25.0-39.0	33.0-45.0	3.3-9.7	6.7-7.1
(N)	(6)	(6)	(6)	(195)	(6)
Northern pike					
$\bar{x}$ ± S.D.	3.3±1.0	35.6±4.9	37.8±5.6	9.0±0.6	-
Range	1.9-4.8	31.9-43.0	31.0-46.0	7.5-10.4	6.7-7.0
(N)	(8)	(8)	(8)	(84)	(8)
Walleye					
$\bar{x}$ ± S.D.	3.4±0.8	33.8±1.8	38.5±4.2	9.5±0.6	-
Range	2.9-4.6	31.9-36.0	32.0-46.0	8.5-10.9	6.8-7.2
(N)	(4)	(4)	(4)	(68)	(4)

TABLE 4 - MEANS AND STANDARD DEVIATIONS ($\bar{x}$ ± S.D.), RANGES AND NUMBER OF OBSERVATIONS (N) OF MEASURED WATER QUALITY PARAMETERS DURING EXPOSURE OF EGGS AND FRY OF SELECTED FRESHWATER FISH SPECIES TO COPPER IN "SOFT" AND "HARD" WATER

Species and type water	Parameter				
	Acidity (mg/l)	Alkalinity (mg/l as $CaCO_3$)	Hardness (mg/l as $CaCO_3$)	Dissolved O_2 (mg/l)	pH
Brook trout					
soft water					
$\bar{x}$ ± S.D.	3.3±1.7	27.8±3.8	37.5±7.3	10.0±0.7	-
Range	1.9-7.0	22.0-31.9	32.0-51.0	87.0-11.1	6.6-7.1
(N)	(8)	(8)	(8)	(24)	(8)
hard water					
$\bar{x}$ ± S.D.	8.3±2.1	177.6±30.4	187.0±22.0	11.0±1.2	-
Range	7.0-11.0	150.7-204.0	167.0-208.0	9.5-13.2	6.7-7.1
(N)	(8)	(8)	(8)	(28)	(8)
Channel catfish					
soft water					
$\bar{x}$ ± S.D.	3.2±0.5	34.1±1.8	36.0±1.1	7.3±2.4	-
Range	2.9-3.8	31.9-36.3	35.0-37.0	6.3-10.6	7.4-7.6
(N)	(6)	(6)	(6)	(100)	(6)
hard water					
$\bar{x}$ ± S.D.	4.8±1.6	172.9±34.6	186.3±38.7	8.7±0.9	-
Range	2.9-6.7	130.9-210.0	136.0-229.0	6.2-10.6	7.5-7.8
(N)	(6)	(6)	(6)	(48)	(6)
Walleye					
soft water					
$\bar{x}$ ± S.D.	3.3±0.7	34.0±1.9	35.0±1.8	9.9±0.7	-
Range	2.9-4.0	31.0-39.0	30.0-39.0	7.8-11.2	6.8-7.3
(N)	(4)	(4)	(4)	(68)	(4)
hard water					
$\bar{x}$ ± S.D.	4.6±1.7	176.0±30.0	189.0±33.0	8.9±0.8	-
Range	2.9-6.8	151.0-209.0	170.0-238.0	7.9-10.1	7.0-7.3
(N)	(4)	(4)	(4)	(33)	(4)

TABLE 5 - MEANS AND STANDARD DEVIATIONS ($\bar{x}$ ± S.D.), RANGES AND NUMBER OF OBSERVATIONS (N) OF MEASURED WATER QUALITY PARAMETERS DURING EXPOSURE OF EGGS AND FRY OF SELECTED FRESHWATER FISH SPECIES TO CADMIUM IN "SOFT" AND "HARD" WATER

	Parameter				
Species and type water	Acidity (mg/l)	Alkalinity (mg/l as $CaCO_3$)	Hardness (mg/l as $CaCO_3$)	Dissolved O_2 (mg/l)	pH
Brook trout					
soft water					
$\bar{x}$ ± S.D.	3.5±1.8	30.0±1.4	37.0±7.2	10.0±0.8	-
Range	1.9-7.0	28.0-32.0	33.0-51.0	8.6-11.0	6.5-7.2
(N)	(8)	(8)	(8)	(37)	(10)
hard water					
$\bar{x}$ ± S.D.	8.1±7.0	177.0±32.0	188.0±27.0	10.6±1.5	-
Range	6.7-9.5	148.0-205.0	164.0-213.0	8.3-12.7	6.7-7.1
(N)	(6)	(6)	(6)	(33)	(6)
Channel catfish					
soft water					
$\bar{x}$ ± S.D.	3.0±0.7	34.0±1.6	37.0±1.3	7.6±0.9	-
Range	1.9-3.8	33.0-36.0	35.0-38.0	5.6-9.8	7.5-7.6
(N)	(6)	(6)	(6)	(91)	(6)
hard water					
$\bar{x}$ ± S.D.	4.8±1.0	172.0±33.4	185.0±35.0	8.6±0.5	-
Range	3.8-5.7	132.0-207.0	142.0-223.0	7.4-9.3	7.7-7.8
(N)	(6)	(6)	(6)	(48)	(6)
Walleye					
soft water					
$\bar{x}$ ± S.D.	3.8±1.6	33.0±1.3	35.0±1.2	10.5±1.1	-
Range	2.9-5.8	31.9-35.0	32.0-39.0	7.7-10.6	6.8-7.3
(N)	(4)	(4)	(4)	(68)	(4)
hard water					
$\bar{x}$ ± S.D.	4.8±1.9	172.0±33.1	187.0±36.0	8.8±0.9	-
Range	1.9-7.0	154.0-210.0	164.0-240.0	7.6-10.3	6.9-7.3
(N)	(4)	(4)	(4)	(34)	(4)

TABLE 6 - MEANS AND STANDARD DEVIATIONS ($\bar{x}$ ± S.D.), RANGES AND NUMBER (N) OF MEASURED CONCENTRATIONS OF LEAD DURING CONTINUOUS EXPOSURE OF EGGS AND FRY OF SELECTED FRESHWATER FISH SPECIES

Species and nominal conc. (μg/1)	Measured conc. (μg/1)			Species and nominal conc. (μg/1)	Measured conc. (μg/1)		
	$\bar{x}$ ± S.D.	Range	(N)		$\bar{x}$ ± S.D.	Range	(N)
Rainbow trout				Channel catfish			
1000	672±108	530-830	8	500	460±14	450-470	4
500	443± 22	410-460	6	250	280±28	260-300	4
250	250± 8	240-260	4	125	136±19	123-150	4
125	146± 29	110-190	6	62	75±15	61- 98	4
62	71± 14	56- 88	8	31	33± 6	24- 43	4
31	49± 11	30- 68	4	16	17± 6	10- 23	4
Lake trout				Bluegill			
1000	483±130	360-720	12	500	447±31	420-490	4
500	404± 68	310-490	8	250	277±25	250-310	4
250	198± 35	150-230	6	125	120± 8	110-130	4
125	120± 19	94-150	10	62	70±20	43- 90	4
62	83± 15	57-110	10	31	33±21	15- 60	4
31	48± 15	34- 68	4	16	12± 6	8- 20	4
Northern pike							
White sucker[a]				Walleye			
500	483± 15	470-500	4	500	397±40	360-440	3
250	253± 17	230-270	4	250	237±51	180-280	3
125	119± 19	94-140	4	125	108±13	94-120	3
62	67± 8	56- 74	4	62	49±16	32- 63	3
31	33± 5	26- 39	4	31	29±11	18- 39	3
				15	22± 6	17- 30	3

[a] Tests run concurrently from same 4-liter dilution apparatus

TABLE 7 - MEANS AND STANDARD DEVIATIONS ($\bar{x}$ ± S.D.), RANGES AND NUMBER (N) OF MEASURED CONCENTRATIONS OF CHROMIUM DURING CONTINUOUS EXPOSURE OF EGGS AND FRY OF SELECTED FRESH-WATER FISH SPECIES

Species and nominal conc. (μg/l)	Measured conc. (μg/l)			Species and nominal conc. (μg/l)	Measured conc. (μg/l)		
	$\bar{x}$ ± S.D.	Range	(N)		$\bar{x}$ ± S.D.	Range	(N)
Rainbow trout							
Lake trout[a]				Channel catfish			
750	822±87	700-940	5	1250	1290±14	1280-1300	4
375	384±42	330-410	5	620	570±28	550- 590	4
187	194±32	140-220	5	310	305±35	280- 330	4
94	105±12	90-120	5	155	150±16	137- 179	4
47	51±13	30- 60	5	77	73± 3	71- 76	4
				39	39± 2	36- 42	4
Northern pike							
White sucker[a]				Bluegill			
2000	1975±125	1800-2100	5	1000	1122±97	980-1200	4
1000	963±125	800-1100	5	500	522±38	470- 560	4
500	538±133	410- 710	5	250	265±13	250- 280	4
250	290± 57	240- 370	5	125	140±14	120- 150	4
125	123± 9	110- 130	5	62	70± 1	68- 70	4
				31	57±35	20- 100	4
Walleye							
2000	2167±153	2000-2300	4				
1000	1125±189	1000-1400	4				
500	558± 74	490- 660	4				
250	288± 44	250- 350	4				
125	133± 35	110- 170	4				
62	80± 24	59- 110	4				

[a] Tests run concurrently from same 4-liter dilution apparatus

TABLE 8 - MEANS AND STANDARD DEVIATIONS ($\bar{x}$ ± S.D.), RANGES AND NUMBER (N) OF MEASURED CONCENTRATIONS OF COPPER DURING CONTINUOUS EXPOSURE OF EGGS AND FRY OF SELECTED FRESHWATER FISH SPECIES IN "SOFT" AND "HARD" WATERS

Soft Water				Hard Water			
Species and nominal conc. (μg/l)	Measured conc. (μg/l)			Species and nominal conc. (μg/l)	Measured conc. (μg/l)		
	$\bar{x}$ ± S.D.	Range	(N)		$\bar{x}$ ± S.D.	Range	(N)
Brook trout				Brook trout			
100	95±7.4	73-107	7	100	74±9.4	60-81	7
50	51±5.1	24- 55	7	50	49±7.0	43-58	7
25	27±3.2	13- 28	7	25	21±5.1	15-27	7
12	13±2.1	7- 14	7	12	13±3.6	8-18	7
6	7±1.6	5- 8	7	6	8±2.3	4-10	7
3	5±1.2	3-5.5	7	3	5±1.6	3- 7	7
Channel catfish				Channel catfish			
25.0	21±4.5	16-27	4	100	66±8.7	53-73	4
12.5	15±3.6	10-18	4	50	34±6.4	28-43	4
6.2	9±2.1	6-11	4	25	19±6.5	11-26	4
3.1	4±1.5	2- 6	4	12	13±5.1	8-17	4
1.6	3±0.7	2- 3	4	6	10±9.4	6-28	4
0.8	0.5±0.3	0.2-0.7	4	3	7±1.9	6-11	4
Walleye				Walleye			
100	91±7.1	86-96	2	100	71±3.5	68-73	2
50	47	-	2	50	38±7.8	32-43	2
25	21±2.1	19-22	2	25	24±2.8	22-26	2
12	13±4.5	10-16	2	12	17	-	2
6	8±2.6	6-10	2	6	14±1.4	13-15	2
3	3±1.1	2- 4	2	3	9±0.7	8.6-9.6	2

TABLE 9 - MEANS AND STANDARD DEVIATIONS ($\bar{x}$ ± S.D.), RANGES AND NUMBER (N) OF MEASURED CONCENTRATIONS OF CADMIUM DURING CONTINUOUS EXPOSURE OF EGGS AND FRY OF SELECTED FRESH-WATER FISH SPECIES IN "SOFT" AND "HARD" WATERS

Soft Water				Hard Water			
Species and nominal conc. (µg/l)	Measured conc. (µg/l)			Species and nominal conc. (µg/l)	Measured conc. (µg/l)		
	$\bar{x}$ ± S.D.	Range	(N)		$\bar{x}$ ± S.D.	Ranges	(N)
Brook trout				Brook trout			
50.0	47±4.8	41-52	4	100	91±12	73-101	4
25.0	24±2.6	20-26	4	50	50±11	37- 59	4
12.5	10±1.2	9-12	4	25	21± 3	20- 25	4
6.2	6.4±0.1	6.4-6.6	4	12	12± 3	10- 16	4
3.1	3.2±0.5	2.5-3.6	4	6	7± 1	6- 9	4
1.5	2.0±0.1	1.8-2.2	4	3	3±0.4	2.6-3.6	4
Channel catfish				Channel catfish			
60.0	54±7.0	44-63	5	100	59±18	52-96	5
30.0	32±5.1	20-33	5	50	33± 8	30-50	5
15.0	20±5.9	7-21	5	25	17± 2	16-21	5
7.5	17±6.7	4-19	5	12	12± 3	7-14	5
3.8	11±4.7	2-13	4	6	5± 2	2- 7	5
1.9	6±2.3	2.6-6.9	4	3	2±0.5	1.1-2.5	5
Walleye				Walleye			
50.0	55±2.6	52-57	3	100	86.7±10.7	75-96	3
25.0	24.7±3.2	20-26	3	50	44.3± 6.8	39-52	3
12.5	8.6±2.1	7.4-11	3	25	19.0± 2.6	16-21	3
6.2	3.7±1.2	2.3-4.7	3	12	8.4± 2.3	6.6-11	3
3.1	1.8±0.7	1.3-2.6	3	6	3.4± 2.4	1.6-6.1	3
1.5	0.9		3	3	1.3± 0.2	1.1-1.5	3

TABLE 10 - MEAN PERCENTAGE HATCH OF EGGS, MEAN SURVIVAL, TOTAL LENGTHS AND WET WEIGHT OF RAINBOW TROUT (Salmo gairdneri) FRY CONTINUOUSLY EXPOSED TO LEAD IN SOFT WATER (34 ± 3.2 mg/l as $CaCO_3$)

Mean measured lead conc. (µg/l)		Mean hatch (%)	1-30 Days		31-60 Days		
			Survival (%)	Mean total length (mm)	Survival (%)	Mean total length (mm)	Mean total wet wt. (mg)
672	A	28	18	20 (1.0)	0	-	-
	B	29[a]	26[a]	20 (0.9)[a]	0	-	-
443	A	96	2	21 (1.1)	0	-	-
	B	87	14[a]	20 (1.5)[a]	0	-	-
250	A	88	24	23 (3.0)	4	38	700
	B	87	12[a]	24 (3.1)[a]	0	-	-
146	A	91	86	28 (2.0)	56	39 (3.1)	710
	B	92	82	28 (1.0)	52[a]	38 (3.0)	690
71	A	90	98	29 (2.2)	84	40 (4.1)	820
	B	87	98	27 (1.6)	96	37 (3.2)	650
49	A	93	90	28 (1.6)	96	38 (3.9)	680
	B	94	90	28 (1.5)	100	37 (4.9)	660
Control	A	87	96	29 (1.7)	96	40 (4.8)	750
	B	82	96	29 (1.7)	92	38 (3.8)	670

[a] Denotes values significantly lower than the controls (Duncan's Multiple Range P=0.05)

Approximately 28% and 12% of rainbow trout fry exposed to 672 and 443 μg/l lead, respectively, exhibited scoliosis (a lateral curvature of the spine) during the initial 30 day post-hatch exposure.

Based on these data, the MATC for lead and rainbow trout eggs and fry is estimated to be between 71 and 146 μg/l.

Lake trout

No significant effect on the percentage of lake trout eggs successfully hatching was observed during continuous exposure to lead concentrations as high as 483 μg/l (Table 11). The reduced hatch observed among eggs exposed to 83 μg/l and among the A replicate of control eggs was apparently due to fungus. Survival of lake trout fry exposed to 483 and 404 μg/l lead for 30 days was significantly lower than survival among control fry and those exposed to lesser concentrations of lead. In addition, growth among fry exposed to 438 and 404 μg/l lead was also significantly reduced when compared to controls.

No lake trout fry survived 60 days exposure to 483 and 404 μg/l lead and survival was significantly reduced among fry exposed for 60 days to 198, 120 and 83 μg/l lead. Survival in the latter three concentrations had not been affected during the initial 30 days exposure indicating a cumulative toxic effect of lead at these concentrations. No statistically significant reduction in total length or wet weight was observed among surviving fish although fry exposed to 198 μg/l lead were smaller than all other fry.

The scoliosis condition observed in rainbow trout exposed to 672 and 443 μg/l lead was not observed among lake trout exposed to a similar (483 μg/l) concentration.

Based on these data, the MATC for lead and lake trout eggs and fry is estimated to be between 48 and 83 μg/l.

Channel catfish

Percentage hatch among catfish eggs in duplicates continuously exposed to 460 μg/l were 93 and 88% indicating no significant effects of exposure on the hatching process (Table 12). Hatching success was variable among other treatments and poor among controls presumably due to an obvious fungus infection. None of the catfish fry continuously exposed to 460 and 280 μg/l lead survived 30 days continuous exposure. In addition, 30 day exposure to 136 μg/l lead significnatly

TABLE 11 - MEAN PERCENTAGE HATCH OF EGGS, MEAN SURVIVAL, TOTAL LENGTHS AND WET WEIGHT OF LAKE TROUT (*Salvelinus namaycush*) FRY CONTINUOUSLY EXPOSED TO LEAD IN SOFT WATER (32.6 ± 3.9 mg/l as $CaCO_3$)

Mean measured lead conc. (µg/l)		Mean hatch (%)	1-30 Days		31-60 Days		
			Survival (%)	Mean total length (mm)	Survival (%)	Mean total length (mm)	Mean total wet wt. (mg)
483	A	78	22	22 (1.1)	0	-	-
	B	83	36[a]	20 (1.1)[a]	0[a]	-	-
404	A	80	16	21 (1.6)	0[a]	-	-
	B	82	16[a]	21 (1.3)[a]	0[a]	-	-
198	A	92	68	24 (1.8)	58	27 (2.8)	120
	B	84	86	25 (1.3)	54[a]	26 (1.7)	130
120	A	66	88	25 (1.8)	56	28 (2.4)	180
	B	66	76	25 (1.8)	58[a]	27 (2.2)	130
83	A	34	90	26 (1.5)	72	29 (2.0)	170
	B	22	86	24 (1.6)	80[a]	28 (2.6)	150
48	A	71	86	26 (2.2)	96	31 (2.5)	200
	B	81	88	26 (2.5)	96	30 (2.8)	190
Control	A	38	64	25 (1.5)	96	30 (2.9)	190
	B	82	68	27 (1.4)	92	30 (2.8)	170

[a] Denotes values significantly lower than the controls (Duncan's Multiple Range P=0.05)

TABLE 12 - MEAN PERCENTAGE HATCH OF EGGS, MEAN SURVIVAL, TOTAL LENGTHS AND WET WEIGHT OF CHANNEL CATFISH (Ictalurus punctatus) FRY CONTINUOUSLY EXPOSED TO LEAD IN SOFT WATER (36.0 ± 1.0 mg/l as $CaCO_3$)

Mean measured lead conc. (µg/l)		Mean hatch (%)	1-30 Days		31-60 Days		
			Survival (%)	Mean total length (mm)	Survival (%)	Mean total length (mm)	Mean total wet wt.(mg)
460	A	93	0	-	-	-	-
	B	88	0[a]	-	-	-	-
280	A	97	0	-	-	-	-
	B	59	0[a]	-	-	-	-
136	A	97	12	18 (1.4)	79	24 (3.3)[a]	150[a]
	B	66	7[a,b]	18 (1.4)	-	-	-
75	A	96	53	20 (2.2)	90	30 (2.9)	250
	B	68	50	19 (3.1)	92	27 (4.3)	210
33	A	54	63	20 (2.3)	84	29 (3.5)	250
	B	64	62	19 (2.8)	76	28 (4.4)	230
17	A	48	39	21 (2.7)	95	31 (5.3)	230
	B	71	48	21 (2.5)	96	32 (4.3)	230
Control	A	21	70	19 (2.3)	84	29 (4.5)	240
	B	20	60	19 (2.2)	76	29 (4.7)	240

[a] Denotes values significantly lower than the controls (Duncan's Multiple Range P=0.05)

[b] All survivors pooled into one experimental unit after 30 days exposure to maintain comparability of numbers of fry/chamber

reduced survival of catfish fry. Continuous exposure to lead for 30 days had no significant effect on total length of surviving catfish.

During days 31-60 of exposure to lead, survival of remaining catfish was similar in all treatments. However, continuous exposure to 136 µg/l of lead for 60 days significantly reduced total length and wet weight of catfish when compared to controls and fish in lower treatments.

Based on these data, the MATC for channel catfish and lead is estimated to be between 75 and 136 µg/l.

Bluegill

Continuous exposure to lead concentrations as high as 447 µg/l had no significant effect on the percentage of bluegill eggs successfully hatching (Table 13). Survival of bluegill fry during the first 30 days exposure to lead was generally poor but obvious differences among treatments were observed. None of the bluegill fry exposed to 447 and 277 µg/l of lead survived for the duration of this period and survival was significantly lower among fry exposed to 120 µg/l than among controls and bluegill exposed to lower concentrations of lead. Prior to death, bluegill exposed to 447 and 277 µg/l exhibited some incidence of scoliosis. In addition, mean total length of surviving bluegill exposed to 120 µg/l of lead was significantly less than that observed for all other groups where bluegill survived.

During the period of 31-60 days exposure, survival of bluegill fry exposed to 120 µg/l was again significantly reduced when compared to other treatments and controls. Also, after 60 days exposure to this concentration, total length and weight of bluegill fry was significantly lower than all other treatments and controls.

Based on these data, the MATC of lead for bluegill eggs and fry is estimated to be between 70 and 120 µg/l.

White sucker

The percentage of white sucker eggs successfully hatching was similar for eggs incubated in controls and in lead concentrations as high as 483 µg/l (Table 14). Only one sucker fry survived 30 days exposure to 483 µg/l lead and survival in one of the replicates of 253 µg/l was lower than generally observed in other treatments. More than half

TABLE 13 - MEAN PERCENTAGE HATCH OF EGGS, MEAN SURVIVAL, TOTAL LENGTHS AND WET WEIGHT OF BLUEGILL (Lepomis macrochirus) FRY CONTINUOUSLY EXPOSED TO LEAD IN SOFT WATER (40.7 ± 7.3 mg/l as $CaCO_3$)

Mean measured lead conc. (μg/l)		Mean hatch (%)	1-30 Days		31-60 Days		
			Survival (%)	Mean total length (mm)	Survival (%)	Mean total length (mm)	Mean total wet wt.(mg)
447	A	97	0	-	-	-	-
	B	94	0[a]	-	-	-	-
277	A	85	0	-	-	-	-
	B	94	0[a]	-	-	-	-
120	A	95	6	19 (3.5)	50	23[a] (3.6)	250
	B	91	6[a]	17[a] (3.1)	75[a]	23 (3.6)	250[a]
70	A	78	12	23 (4.2)	100	29 (1.4)	510
	B	91	16	23 (5.1)	70	27 (4.3)	470
33	A	71	24	21 (2.1)	100	25 (3.6)	360
	B	87	24	21 (4.2)	88	28 (2.3)	420
12	A	65	14	23 (3.4)	100	31 (5.6)	460
	B	95	16	25 (3.1)	100	27 (4.5)	380
Control	A	90	16	24 (2.5)	100	30 (1.7)	410
	B	65	24	21 (2.8)	100	25 (4.4)	360

[a] Denotes values significantly lower than the controls (Duncan's Multiple Range P=0.05)

TABLE 14 - MEAN PERCENTAGE HATCH OF EGGS, MEAN SURVIVAL, TOTAL LENGTHS AND WET WEIGHT OF WHITE SUCKER (*Catostomus commersoni*) FRY CONTINUOUSLY EXPOSED TO LEAD IN SOFT WATER (37.6 ± 5.9 mg/l as $CaCO_3$)

Mean measured lead conc. (μg/l)		Mean hatch (%)	1-30 Days		31-60 Days		
			Survival (%)	Mean total length (mm)	Survival (%)	Mean total length (mm)	Mean total wet wt.(mg)
483	A	65	2	15	0	-	-
	B	70	0[a]	-[a]	0	-	-
253	A	70	6[a]	15 (1.4)	2	18	60
	B	68	20	17 (1.9)[a]	20	22 (4.4)[a]	80[a]
119	A	66	32	18 (1.2)	32	28 (2.7)	140
	B	72	30	19 (1.7)	24	30 (3.8)	230
67	A	80	40	19 (1.4)	36	29 (2.7)	160
	B	78	24	20 (1.6)	24	32 (4.4)	220
33	A	84	22	20 (2.1)	20	33 (2.9)	250
	B	64	24	20 (1.2)	20	32 (2.6)	280
Control	A	63	46	22 (1.5)	32	30 (3.5)	160
	B	65	30	21 (2.8)	22	31 (3.3)	220

[a] Denotes values significantly lower than the controls (Duncan's Multiple Range P=0.05)

of the sac fry of white sucker exposed to 483 and 253 µg/l lead exhibited severe scoliosis shortly after hatching and expired before reaching the swim-up stage. Survival was generally low during the initial 30 days due to difficulties encountered in feeding sucker fry with both live food and trout starter. Total length of surviving sucker fry after 30 days exposure to 483 and 253 µg/l lead was significantly less than that observed for control fry. Survival of fry after 60 days, except for one of the duplicates at 253 µg/l previously mentioned, was not significantly different, statistically, between controls and lead exposed fry.

Total lengths and wet weights of sucker fry exposed to 253 µg/l lead for 60 days were significantly less than lengths and weights of control fry and those exposed to lesser concentrations of lead.

Based on these data, the MATC for white sucker and lead is estimated to be between 119 and 253 µg/l.

Northern pike

The percentage of successful hatching was similar for northern pike eggs incubated in controls and in lead concentrations as high as 483 µg/l (Table 15). After 20 days exposure, survival was significantly reduced among pike fry exposed to 483 µg/l lead when compared with controls and lesser concentrations of lead. Approximately 20% of fry exposed to 483 µg/l lead exhibited severe scoliosis after one week of exposure.

All data recorded after 20 days exposure are highly variable due to difficulty in controlling cannibalism among pike fry. Northern pike hatched and fed in water of 18°C were found to accept invertebrates as food for only one week after which we observed cannibalism despite the presence of large quantities of Daphnia in the growth chamber. During the initial 20 days after hatching, cannibalism was somewhat controlled by the constant maintenance of a supply of white sucker or fathead minnow fry in the growth chamber with young pike. After 20 days, the food size preference of young pike apparently differed from the supply of forage fish which was available and cannibalism masked any toxicant induced reduction in survival or growth.

Based on the reduced survival and incidence of scoliosis in hatchery pike exposed for 20 days to 483 µg/l lead, the MATC for this species is estimated to be between 253 and 483 µg/l.

TABLE 15 - MEAN PERCENTAGE HATCH OF EGGS, MEAN SURVIVAL, TOTAL LENGTHS AND WET WEIGHT OF NORTHERN PIKE (*Esox lucius*) FRY CONTINUOUSLY EXPOSED TO LEAD IN SOFT WATER (35.4 ± 4.8 mg/l as $CaCO_3$)

Mean measured lead conc. (µg/l)	Mean hatch (%)	1-20 Days		21-50 Days		
		Survival (%)	Mean total length (mm)	Survival (%)	Mean total length (mm)	Mean total wet wt. (mg)
483 A	72	18	19 (2.1)	0	-	-
B	76	24[a]	30 (4.0)	8	57 (12.2)	1310
253 A	73	40	33 (6.5)	6	77 (10.3)	2960
B	88	46	38 (5.0)	6	81 (14.0)	3310
119 A	72	48	36 (5.1)	10	67 (11.3)	1840
B	73	40	39 (6.1)	16	61 (6.5)	1200
67 A	80	60	33 (7.7)	18	65 (10.6)	1610
B	83	40	42 (6.5)	14	64 (10.1)	1460
33 A	70	56	35 (6.9)	8	74 (9.2)	2790
B	83	48	37 (6.0)	14	62 (15.0)	1700
Control A	80	34	31 (8.1)	16	65 (9.7)	1760
B	79	38	37 (6.6)	10	74 (10.2)	2680

[a] Denotes values significantly lower than the controls (Duncan's Multiple Range P=0.05)

Walleye

Exposure to lead concentrations as high as 397 µg/l had no significant effect on the percentage of walleye eggs which hatched successfully (Table 16). Percentage survival of walleye fry after 30 days exposure to 397 µg/l lead appeared reduced when compared to controls and lower lead concentrations, however, variability between replicates and the generally poor success in feeding walleye fry precluded ascribing statistical significance to this observation. Many of the walleye fry exposed to 397 µg/l lead exhibited the scoliosis which was symptomatic of lead toxicity for other species discussed previously.

TABLE 16 - MEAN PERCENTAGE HATCH OF EGGS, MEAN SURVIVAL AND TOTAL LENGTHS OF WALLEYE (Stizostedion vitreum) FRY CONTINUOUSLY EXPOSED TO LEAD IN SOFT WATER (37.6 ± 5.2 mg/l as $CaCO_3$)

Mean measured lead conc. (µg/l)		Mean hatch (%)	1-30 Days	
			Survival (%)	Mean total length (mm)
397	A	70	0	-
	B	69	12	10 (2.4)
237	A	64	16	11 (2.2)
	B	65	16	11 (2.6)
108	A	68	26	8 (1.8)
	B	61	26	11 (3.6)
49	A	67	18	11 (2.9)
	B	66	32	12 (1.8)
29	A	58	24	11 (3.1)
	B	73	14	10 (2.0)
22	A	66	24	12 (3.0)
	B	59	46	10 (2.5)
Control	A	48	38	13 (1.7)
	B	62	28	13 (1.3)

Based on these data, the MATC of lead for walleye is estimated to be between 237 and 397 µg/l.

EXPOSURE OF FISH TO CHROMIUM

Rainbow trout

Two separate exposures of rainbow trout eggs and fry to chromium were completed. During the first exposure, the mean measured chromium concentrations ranged from 1.6 to 49.7 mg/l (Table 17). None of the trout eggs exposed to 26.7 and 49.7 mg/l hatched successfully. The percentage of eggs successfully hatching among groups exposed to 12.2 and 6.1 mg/l was significantly lower than controls and eggs exposed to lower concentrations of chromium. None of the fry survived 30 days exposure to 12.2 mg/l chromium, and 30 days exposure to 6.1 and 3.2 mg/l significantly reduced survival of trout fry when compared to controls. Mean total lengths of trout fry were significantly less than controls after 30 days exposure to to all chromium concentrations including 1.6 mg/l (the lowest concentration tested). During the period of 31-60 days we observed that the exposure to 1.6 mg/l chromium significantly reduced survival, total length, and total wet weight of rainbow trout fry when compared to controls.

In view of the fact that we could not estimate limits on the MATC based on these data, a second exposure of rainbow trout eggs and fry to chromium was conducted with mean measured chromium concentrations ranging from 51 to 822 µg/l. These concentrations provided a continuum of the 0.5 dilution factor from the first test. As would be expected from the first test, exposure to concentrations of chromium as high as 822 µg/l had no significant effect on the percentage of rainbow trout eggs successfully hatching or on survival of fry during the first 30 days post-hatch exposure (Table 18). However, after 30 days exposure, the total length of trout fry exposed to 822 µg/l was significantly reduced compared with controls and lesser chromium concentrations.

Continuous exposure to 822 µg/l chromium for 60 days significantly reduced survival of trout fry when compared to controls and all other treatments. In addition, total length of fry was significantly less than controls after 60 days exposure to 822 and 384 µg/l chromium. Finally, total weight of fry was significantly lower than controls after 60 days exposure to chromium concentrations $\geq$ 105 µg/l.

Based on these data, the MATC for rainbow trout and chromium is estimated to be between 51 and 105 µg/l.

TABLE 17 - MEAN PERCENTAGE HATCH OF EGGS, MEAN SURVIVAL, TOTAL LENGTHS AND WET WEIGHTS OF RAINBOW TROUT (Salmo gairdneri) FRY CONTINUOUSLY EXPOSED TO CHROMIUM IN SOFT WATER (34.0 ± 3.2 mg/l as $CaCO_3$)

Mean measured chromium conc. (mg/l)		Mean hatch (%)	1-30 Days		31-60 Days		
			Survival (%)	Mean total length (mm)	Survival (%)	Mean total length (mm)	Mean total wet wt. (mg)
49.7	A	0	-	-	-	-	-
	B	0^{a}	-	-	-	-	-
26.7	A	0	-	-	-	-	-
	B	0^{a}	-	-	-	-	-
12.2	A	0	-	-	-	--	-
	B	31^{a}	0^{a}	-	-	-	-
6.1	A	56	7	20 (1.4)	0	-	-
	B	62^{a}	0^{a}	-^{a}	0	-	-
3.2	A	82	17	20 (1.0)	0	-	-
	B	85	4^{a}	19 (1.3)^{a}	0	-	-
1.6	A	77	84	21 (1.4)	14^{a}	21 (1.2)^{a}	250^{a}
	B	87	85	21 (1.5)^{a}	2	28 -	250
Control	A	76	96	26 (1.7)	96	34 (3.8)	480
	B	77	99	26 (1.6)	88	33 (3.3)	460

[a] Denotes values significantly lower than the controls (Duncan's Multiple Range P=0.05)

TABLE 18 - MEAN PERCENTAGE HATCH OF EGGS, MEAN SURVIVAL, TOTAL LENGTHS AND WET WEIGHT OF RAINBOW TROUT (Salmo gairdneri) FRY CONTINUOUSLY EXPOSED TO CHROMIUM IN SOFT WATER (33.4 ± 4.5 mg/l as $CaCO_3$)

Mean measured chromium conc. (µg/l)	Mean hatch (%)	1-30 Days		31-60 Days		
		Survival (%)	Mean total length (mm)	Survival (%)	Mean total length (mm)	Mean total wet wt. (mg)
822 A	72	94	23 (1.5)	20	25 (3.6)	170
B	70	95	23 (2.0)[a]	22[a]	25 (3.8)[a]	170[a]
384 A	68	96	26 (1.4)	78	32 (3.3)	270
B	79	99	26 (1.3)	86	33 (2.9)[a]	300[a]
194 A	66	100	27 (1.4)	96	35 (4.1)	350
B	68	97	28 (1.4)	96	37 (3.5)	380[a]
105 A	70	100	27 (1.5)	88	37 (3.8)	400
B	68	99	27 (1.6)	80	35 (3.3)	370[a]
51 A	66	99	28 (1.8)	88	38 (2.8)	450
B	72	98	26 (1.7)	86	37 (3.3)	440
Control A	69	98	28 (2.3)	88	36 (4.6)	450
B	76	91	26 (2.1)	92	38 (4.4)	550

[a] Denotes values significantly lower than the controls (Duncan's Multiple Range P=0.05)

Lake trout

As with the rainbow trout, two separate exposures of lake trout eggs and fry to chromium were conducted. During the first exposure, mean measured concentrations of chromium ranged from 1.4 to 50.7 mg/l (Table 19). No lake trout were observed to hatch successfully during exposure to 50.7 mg/l and the percentage of lake trout eggs successfully hatching was severely reduced by exposure to 24.4 mg/l chromium. None of the lake trout survived 30 days exposure to 24.4 mg/l chromium, and survival of lake trout was significantly reduced as a result of 30 days exposure to 11.6 and 6.0 mg/l chromium when compared to controls. After 30 days exposure, mean lengths of lake trout were significantly lower in all chromium concentrations, including 1.4 mg/l, when compared with controls.

After 60 days exposure to chromium, survival of lake trout fry appeared to be reduced in all chromium concentrations when compared with controls. Analysis of variance and Duncan's Multiple Range Tests indicated that survival was significantly reduced among lake trout fry exposed to 11.6 and 6.0 mg/l chromium but that variability between replicates precluded ascribing statistical significance to the apparent reduced survival among fry exposed to 1.4 and 2.9 mg/l chromium. Total lengths and total wet weights of lake trout after 60 days exposure were significantly lower in all chromium concentrations when compared with controls.

The second exposure of lake trout eggs and fry to chromium was conducted concurrently with the second rainbow trout exposure using mean measured concentrations ranging from 51 to 822 µg/l. Percentage hatch of lake trout eggs was sub-standard in all chromium concentrations and controls, but generally indicated no adverse effects occurred during exposure to concentrations as high as 822 µg/l (Table 20). After 30 days exposure, percentage survival and total lengths of lake trout fry were similar for all test concentrations of chromium and controls.

After 60 days exposure,survival and total lengths of lake trout were again similar for controls and chromium concentrations tested. At termination the mean total weights of lake trout indicated significant differences due to treatment and a Duncan's Multiple Range Test indicated that total weights of lake trout exposed to 822, 384 and 194 µg/l chromium were significantly lower than those from controls and other treatments.

TABLE 19 - MEAN PERCENTAGE HATCH OF EGGS, MEAN SURVIVAL, TOTAL LENGTHS AND WET WEIGHT OF LAKE TROUT (Salvelinus namaycush) FRY CONTINUOUSLY EXPOSED TO CHROMIUM IN SOFT WATER (33.0 ± 3.9 mg/l as $CaCO_3$)

Mean measured chromium conc. (mg/l)		Mean hatch (%)	1-30 Days		31-60 Days		
			Survival (%)	Mean total length (mm)	Survival (%)	Mean total length (mm)	Mean total wet wt. (mg)
50.7	A	0	-	-	-	-	-
	B	0[a]	-	-	-	-	-
24.4	A	2	0	-	-	-	-
	B	7[a]	0[a]	-	-	-	-
11.6	A	46	28	21 (0.8)	3	22	90
	B	39	38[a]	21 (1.0)[a]	13[a]	22 (1.6)[a]	90[a]
6.0	A	60	35	21 (1.0)	0	-	-
	B	53	49[a]	22 (0.9)[a]	56[a]	22 (1.1)[a]	60[a]
2.9	A	64	39	23 (1.4)	43	22 (1.6)	90
	B	51	60	22 (1.3)[a]	52	22 (1.8)[a]	100[a]
1.4	A	47	38	22 (1.2)	54	22 (1.5)	90
	B	39	60	22 (1.3)[a]	70	24 (2.2)[a]	90[a]
Control	A	40	72	26 (2.0)	94	32 (2.6)	200
	B	38	63	27 (1.9)	84	31 (2.5)	220

[a] Denotes values significantly lower than the controls (Duncan's Multiple Range P=0.05)

TABLE 20 - MEAN PERCENTAGE HATCH OF EGGS, MEAN SURVIVAL, TOTAL LENGTHS AND WET WEIGHT OF LAKE TROUT (*Salvelinus namaycush*) FRY CONTINUOUSLY EXPOSED TO CHROMIUM IN SOFT WATER (34.0 ± 4.8 mg/l as $CaCO_3$)

Mean measured chromium conc. (µg/l)		Mean hatch (%)	30 Days		60 Days		
			Survival (%)	Mean total length (mm)	Survival (%)	Mean total length (mm)	Mean total wet wt. (mg)
822	A	23	71	26 (0.9)	63	28 (1.8)	124
	B	37	97	26 (1.8)	84	27 (6.6)	126[a]
384	A	37	95	26 (1.6)	90	30 (2.5)	151
	B	37	92	26 (2.1)	92	30 (4.3)	135[a]
194	A	38	97	26 (2.5)	96	30 (1.8)	155
	B	34	85	27 (2.4)	88	30 (2.6)	159[a]
105	A	35	90	27 (0.9)	92	31 (1.6)	173
	B	30	90	27 (2.0)	88	30 (2.3)	177
51	A	36	83	27 (1.6)	92	33 (2.0)	178
	B	34	87	27 (2.2)	80	29 (2.3)	175
Control	A	20	76	26 (1.1)	88	31 (1.9)	173
	B	0[b]	-	-	-	-	-

[a] Denotes values significantly lower than the controls (Duncan's Multiple Range P=0.05)

[b] A malfunction of the thermoregulatory apparatus was responsible for the mortality experienced in this duplicate

Based on these data, the MATC for lake trout eggs and fry and chromium is estimated to be between 105 and 194 μg/l.

Channel catfish

Exposure to chromium concentrations as high as 1290 μg/l did not significantly reduce the percentage of channel catfish eggs which hatched successfully (Table 21). The reduced hatch observed in controls and among eggs exposed to 39 μg/l chromium was a result of a fungus infection. After 30 days post-hatch exposure, the percentage survival and total lengths were significantly lower among channel catfish fry exposed to concentrations of chromium $\geq$ 305 μg/l when compared with controls and lesser chromium concentrations.

During the 31-60 days post-hatch exposure to 305 μg/l, survival of remaining catfish fry was similar to survival in controls and lesser chromium concentrations, presumably due to the "vigor" of fish which survived the 0-30 day exposure. None of the channel catfish fry survived 60 days exposure to 1290 μg/l chromium and the survival, total length and wet weight of channel catfish exposed for 60 days to 570 μg/l chromium was significantly lower when compared with controls.

Based on these data, the MATC of chromium for egg and fry stages of channel catfish is estimated to be between 150 and 305 μg/l.

Bluegill

The percentage of bluegill which hatched successfully was similar in controls and among eggs exposed to chromium concentrations as high as 1122 μg/l (Table 22). Percentage survival of bluegill fry 30 days post-hatch was highly variable and reflected the difficulty encountered in starting bluegill fry on food. Total lengths of bluegill fry appeared reduced after 30 days exposure to 1122, 522 and 265 μg/l chromium, however variability between duplicates precluded ascribing statistical significance to these observations.

During the period of 31-60 days exposure, survival of remaining fry was excellent and indicated no significant effect due to exposure to chromium concentrations as high as 1122 μg/l. Total lengths of bluegill after 60 days exposure again appeared to be reduced as a result of exposure to the higher chromium concentrations but differences were not statistically significant due to variability between duplicates.

TABLE 21 - MEAN PERCENTAGE HATCH OF EGGS, MEAN SURVIVAL, TOTAL LENGTHS AND WET WEIGHT OF CHANNEL CATFISH (*Ictalurus punctatus*) FRY CONTINUOUSLY EXPOSED TO CHROMIUM IN SOFT WATER (36.2 ± 1.2 mg/l as $CaCO_3$)

Mean measured chromium conc. (μg/l)	Mean hatch (%)	1-30 Days		31-60 Days		
		Survival (%)	Mean total length (mm)	Survival (%)	Mean total length (mm)	Mean total wet wt.(mg)
1290 A	75	0	-	0	-	-
B	95	5[a]	14 (0.4)[a]	0	-	-
570 A	80	4	16 (1.7)	7	21 (3.0)	120
B	93	12[a]	16 (1.7)[a]	10[a]	21 (3.0)[a]	120[a]
305 A	83	24	18 (2.4)	72	31 (5.0)	290
B	84	13[a]	18 (2.4)[a]	88	24 (4.2)	170
150 A	78	23	22 (2.2)	77	34 (5.1)	320
B	74	51	19 (2.1)	87	28 (4.6)	220
73 A	71	74	23 (2.5)	98	35 (2.8)	340
B	59	60	24 (3.1)	92	36 (3.5)	350
39 A	33	68	24 (3.4)	96	38 (5.4)	380
B	56	70	21 (3.0)	100	34 (3.7)	280
Control A	31	44	22 (3.1)	85	33 (6.8)	360
B	37	62	20 (2.8)	86	31 (4.4)	300

[a] Denotes values significantly lower than the controls (Duncan's Multiple Range P=0.05)

TABLE 22 - MEAN PERCENTAGE HATCH OF EGGS, MEAN SURVIVAL, TOTAL LENGTHS AND WET WEIGHT OF BLUEGILL (Lepomis macrochirus) FRY CONTINUOUSLY EXPOSED TO CHROMIUM IN SOFT WATER (38.3 ± 4.6 mg/l as $CaCO_3$)

Mean measured chromium conc. (μg/l)		Mean hatch (%)	1-30 Days		31-60 Days		
			Survival (%)	Mean total length (mm)	Survival (%)	Mean total length (mm)	Mean total wet wt. (mg)
1122	A	85	38	14 (1.7)	94	20 (2.0)	140
	B	62	28	14 (1.9)	94	18 (2.1)	120[a]
522	A	85	32	16 (3.7)	80	22 (4.6)	230
	B	70	16	20 (3.1)	100	24 (3.9)	250
265	A	88	62	15 (2.2)	90	20 (4.0)	180
	B	83	42	18 (3.6)	55	24 (2.9)	220
140	A	81	18	23 (1.9)	100	27 (3.5)	290
	B	83	24	20 (3.6)	90	26 (1.5)	290
70	A	84	50	17 (3.4)	84	20 (5.0)	180
	B	88	10	24 (2.3)	100	31 (4.4)	320
57	A	89	12	22 (2.5)	100	23 (6.8)	290
	B	86	18	22 (2.5)	92	26 (3.8)	300
Control	A	90	18	25 (4.0)	83	27 (4.4)	320
	B	85	30	22 (3.0)	100	24 (4.0)	290

[a] Denotes values significantly lower than the controls (Duncan's Multiple Range P=0.05)

Total wet weight of bluegill fry exposed for 60 days to 1122 µg/l chromium was significantly lower than that of controls and fry exposed to lesser chromium concentrations. Although this was the only statistically valid effect observed in the test it is supported by trends in total length and the fact that total weight appeared to be the most sensitive indicator of chromium effects on fry of rainbow trout and lake trout.

Based on these data, the MATC for chromium and eggs and fry of bluegill is estimated to be between 522 and 1122 µg/l, however, it is possible that growth effects due to exposure to lesser concentrations were masked by the poor success in early feeding of these fry.

White sucker

The percentage of white sucker eggs which hatched successfully was similar among controls and eggs exposed to chromium concentrations as high as 1975 µg/l (Table 23). After 30 days post-hatch exposure, the percentage survival of white sucker fry was also unaffected by exposure to the range of chromium concentrations tested. However, total length of white sucker fry after 30 days exposure to 1975 µg/l chromium was significantly reduced when compared with controls.

Survival of white sucker fry exposed to any of the chromium concentrations tested was not significantly affected after 60 days. Both total length and total wet weight of white suckers after 60 days exposure to 1975, 963 and 538 µg/l chromium were significantly reduced when compared with controls. Again, the reduction in total weight of fry after 60 days exposure appears to be the most sensitive indicator of sub-lethal effects of chromium.

Based on these data,the MATC of chromium for eggs and fry of white suckers is estimated to be between 290 and 538 µg/l.

Northern pike

Exposure to chromium concentrations as high as 1975 µg/l had no significant effect on the percentage of northern pike eggs which hatched successfully when incubated from the eyed stage (Table 24). After 20 days, a significant reduction in survival of northern pike fry exposed to 1975 and 963 µg/l chromium was observed.

Data on survival and growth of northern pike after 20 days exposure is not considered reliable due to the effect of

TABLE 23 - MEAN PERCENTAGE HATCH OF EGGS, MEAN SURVIVAL, TOTAL LENGTHS AND WET WEIGHT OF WHITE SUCKER (Catostomus commersoni) FRY CONTINUOUSLY EXPOSED TO CHROMIUM IN SOFT WATER (38.8 ± 5.3 mg/l as $CaCO_3$)

Mean measured chromium conc. (µg/l)		Mean hatch (%)	1-30 Days		31-60 Days		
			Survival (%)	Mean total length (mm)	Survival (%)	Mean total length (mm)	Mean total wet wt. (mg)
1975	A	94	86	16 (2.1)	46	18 (2.6)	40
	B	97	54	18 (1.4)^a	10	18 (1.8)^a	40^a
963	A	98	50	20 (2.5)	46	25 (3.6)	130
	B	96	76	20 (2.6)	52	25 (3.7)^a	100^a
538	A	100	64	21 (1.9)	48	27 (3.4)	170
	B	96	44	21 (2.2)	34	28 (1.7)^a	170a
290	A	94	36	23 (2.0)	36	33 (3.2)	260
	B	97	54	22 (2.2)	50	28 (2.9)	160
123	A	93	60	21 (1.8)	46	32 (2.3)	210
	B	93	70	21 (2.5)	52	30 (3.7)	180
Control	A	88	74	20 (1.9)	48	32 (2.5)	260
	B	97	42	21 (1.8)	42	31 (4.1)	230

[a] Denotes values significantly lower than the controls (Duncan's Multiple Range P=0.05)

TABLE 24 - MEAN PERCENTAGE HATCH OF EGGS, MEAN SURVIVAL, TOTAL LENGTHS AND WET WEIGHT OF NORTHERN PIKE (*Esox lucius*) FRY CONTINUOUSLY EXPOSED TO CHROMIUM IN SOFT WATER (37.8 ± 5.6 mg/1 as $CaCO_3$)

Mean measured chromium conc. (µg/1)		Mean hatch (%)	1-20 Days		21-50 Days		
			Survival (%)	Mean total length (mm)	Survival (%)	Mean total length (mm)	Mean total wet wt.(mg)
1975	A	83	14	-	0	-	-
	B	88	24[a]	23 (4.6)	8	40 (9.8)	340
963	A	81	32	26 (3.6)	12	44 (3.6)	440
	B	83	20[a]	-	0	-	-
538	A	83	38	27 (7.5)	8	57 (6.5)	890
	B	90	38	27 (4.5)	12	50 (5.7)	640
290	A	76	34	32 (5.9)	2	70 -	2000
	B	80	34	29 (3.6)	8	58 (5.2)	940
123	A	74	42	31 (3.8)	18	56 (10.2)	990
	B	87	34	34 (4.9)	10	54 (5.4)	780
Control	A	73	48	36 (5.1)	14	54 (7.0)	880
	B	83	48	29 (4.8)	14	56 (10.2)	1180

[a] Denotes values significantly lower than the controls (Duncan's Multiple Range P=0.05)

cannibalism which could not be controlled after pike were 20 days old.

The MATC of chromium for northern pike eggs and fry is estimated to be between 538 and 963 µg/l although effects of lower concentrations on survival and growth may have been masked by the cannibalism among pike fry in the growth chambers.

Walleye

Exposure to chromium concentrations as high as 2167 µg/l had no significant effect on the percentage of walleye eggs which hatched successfully (Table 25). Survival of walleye fry after 30 days exposure was low in all treatments but indicated no significant effects of exposure to the range of chromium concentrations which were tested. Again, the generally poor success in feeding walleye fry is responsible for the lack of more conclusive data on the toxicity of chromium to this species. Based on the data available, the MATC for chromium and walleyes is estimated to be >2161 µg/l.

TABLE 25 - MEAN PERCENTAGE HATCH OF EGGS, MEAN SURVIVAL AND TOTAL LENGTHS OF WALLEYE (Stizostedion vitreum) FRY CONTINUOUSLY EXPOSED TO CHROMIUM IN SOFT WATER (38.5 ± 4.2 mg/l as $CaCO_3$)

Mean measured chromium conc. (µg/l)		Mean hatch (%)	1-30 Days	
			Survival (%)	Mean total length (mm)
2167	A	63	18	10 (3.7)
	B	65	20	8 (2.2)
1125	A	71	32	12 (2.9)
	B	73	30	11 (2.6)
558	A	73	20	10 (2.7)
	B	65	28	10 (2.5)
288	A	73	28	11 (3.3)
	B	65	24	11 (3.5)
133	AA	58	10	8 (3.8)
	B	63	14	10 (1.4)
80	A	57	14	10 (4.2)
	B	67	24	9 (2.1)
Control	A	62	10	10 (2.5)
	B	69	14	10 (2.0)

[a] Denotes values significantly lower than the controls (Duncan's Multiple Range P=0.05)

EXPOSURE OF FISH TO COPPER

Brook trout (soft water)

None of the brook trout eggs exposed to 95 µg/l of copper in soft water (37.5 mg/l as $CaCO_3$) hatched successfully (Table 26). Exposure to 51, 27 and 13 µg/l copper, significantly reduced the percentage hatch of brook trout eggs when compared to controls and eggs exposed to lower concentrations of copper. Concentrations of 51 and 27 µg/l copper significantly reduced survival of brook trout fry when compared to other treatments and controls after 30 days exposure. Finally, total lengths among fry exposed to 51, 27 and 13 µg/l copper for 30 days were significantly less than controls and those at lower concentrations.

Continuous exposure for 60 days to concentrations of copper as high as 13 µg/l had no significant effect on survival of brook trout fry. However, exposure to copper concentrations $\geq$ 5 µg/l significantly reduced total length and wet weight of fry when compared to controls.

The mean concentration of copper in the diluent water (control) was measured to be 3 µg/l. Thus, the MATC of copper for brook trout in soft water (37.5 mg/l as $CaCO_3$) is estimated to be between 3 and 5 µg/l.

Brook trout (hard water)

The percentage hatch of brook trout eggs was significantly reduced by exposure to 74 µg/l copper in hard water (187 mg/l as $CaCO_3$) when compared to controls (Table 27). None of the brook trout fry survived 30 days exposure to 74 µg/l copper and both survival and total length were reduced by 30 days exposure to 49 µg/l when compared with controls.

None of the brook trout fry survived 60 days exposure to 49 µg/l copper while survival among fry exposed to lower copper concentrations continued to be comparable with controls. Total length and wet weight of brook trout at the end of exposure (60 days) were similar for controls and fry exposed to 5 µg/l copper but were significantly reduced by exposure to 21, 13 and 8 µg/l where survival was generally excellent.

Based on these data, the MATC of copper for brook trout eggs and fry exposed in hard water (187 mg/l as $CaCO_3$) is estimated to be between 5 and 8 µg/l.

TABLE 26 - MEAN PERCENTAGE HATCH OF EGGS, MEAN SURVIVAL, TOTAL LENGTHS AND WET WEIGHT OF BROOK TROUT (Salvelinus fontinalis) FRY CONTINUOUSLY EXPOSED TO COPPER IN SOFT WATER (37.5 ± 7.3 mg/l as $CaCO_3$)

Mean measured copper conc. (μg/l)	Mean hatch (%)	1-30 Days		31-60 Days		
		Survival (%)	Mean total length (mm)	Survival (%)	Mean total length (mm)	Mean total wet wt. (mg)
95 A	0	-	-	-	-	-
B	0[a]	-	-	-	-	-
51 A	5	0	-	-	-	-
B	2[a]	2[a]	18 (1.5)[a]	0	-	-
27 A	6	10	19 (1.8)	8	18 (1.2)	80
B	4[a]	6[a]	18 (1.9)[a]	6[a]	17 (3.8)[a]	96[a]
13 A	43	96	19 (1.8)	96	22 (7.1)	120
B	25[a]	74	18 (1.5)[a]	72	23 (2.4)[a]	101[a]
7 A	63	100	22 (2.0)	98	24 (3.0)	126
B	72	98	21 (1.7)	98	24 (4.2)[a]	127[a]
5 A	72	100	22 (2.3)	98	26 (3.3)	144
B	77	98	22 (1.9)	90	25 (4.0)[a]	151[a]
Control A	79	100	22 (2.2)	96	28 (5.3)	192
B	73	100	23 (2.3)	98	29 (4.2)	240

[a] Denotes values significantly lower than the controls (Duncan's Multiple Range P=0.05)

TABLE 27 - MEAN PERCENTAGE HATCH OF EGGS, MEAN SURVIVAL, TOTAL LENGTHS AND WET WEIGHT OF BROOK TROUT (*Salvelinus fontinalis*) FRY CONTINUOUSLY EXPOSED TO COPPER IN HARD WATER (187.0 ± 22.0 mg/1 as $CaCO_3$)

Mean measured copper conc. (µg/1)		Mean hatch (%)	1-30 Days		31-60 Days		
			Survival (%)	Mean total length (mm)	Survival (%)	Mean total length (mm)	Mean total wet wt. (mg)
74	A	19	0	-	-	-	-
	B	12[a]	0[a]	-	-	-	-
49	A	35	52	17 (1.6)	0	-	-
	B	35	70[a]	17 (1.0)[a]	0[a]	-	-
21	A	45	100	19 (1.8)	76	19 (2.6)	47
	B	44	90	19 (1.7)	58	19 (1.6)[a]	48[a]
13	A	48	100	20 (1.2)	76	22 (1.3)	81
	B	49	98	20 (1.8)	90	20 (2.3)[a]	78[a]
8	A	49	100	19 (1.3)	74	21 (2.2)	79
	B	53	100	20 (1.4)	86	19 (2.1)[a]	75[a]
5	A	45	100	20 (1.5)	68	24 (2.2)	110
	B	51	100	20 (2.5)	80	22 (2.0)	117
Control	A	56	100	20 (1.7)	58	27 (1.8)	117
	B	39	100	20 (1.4)	100	24 (3.2)	128

[a] Denotes values significantly lower than controls (Duncan's Multiple Range P=0.05)

Channel catfish (soft water)

Exposure to concentrations of copper as high as 24 µg/l in water of 36 mg/l total hardness appeared to have no effect on the percentage of channel catfish eggs which hatched successfully (Table 28). Exposure of catfish fry for 30 days to 24 and 18 µg/l copper significantly reduced both percentage survival and total length when compared with fry in controls and those exposed to lower concentrations of copper.

No catfish fry survived 60 days exposure to 24 µg/l, however, survival of fish exposed to 18 µg/l (during the 31-60 day period of exposure) was not significantly different from controls. This is possibly due to the vigor of individuals which survived the initial 30 day exposure. Total length of catfish exposed for 60 days to 18 µg/l was significantly reduced compared to controls. Total weight of these fry appeared reduced at this time but data were too variable to ascribe statistical significance to this observation.

Based on these data, the MATC of copper for eggs and fry of channel catfish in water of 36 mg/l total hardness is estimated to be between 12 and 18 µg/l.

Channel catfish (hard water)

Percentage hatchability of channel catfish eggs appeared reduced by exposure to 66 µg/l copper in water with a total hardness of 186 mg/l (Table 29), however, variability between duplicates in other treatments precludes ascribing statistical significance to this observation. None of the catfish fry survived 30 days exposure to 66 and 34 µg/l copper, and survival was reduced by exposure to 19 µg/l when compared with controls.

After 60 days exposure, the percentage of channel catfish fry surviving exposure to 19 µg/l copper was significantly less than controls. Total length and wet weight of catfish exposed to this concentration were also significantly less at this time when compared with control fish.

Based on these data, the MATC of copper for channel catfish in hard water (186 mg/l as $CaCO_3$) is estimated to be between 13 and 19 µg/l.

TABLE 28 - MEAN PERCENTAGE HATCH OF EGGS, MEAN SURVIVAL, TOTAL LENGTHS AND WET WEIGHT OF CHANNEL CATFISH (Ictalurus punctatus) FRY CONTINUOUSLY EXPOSED TO COPPER IN SOFT WATER (36.0 ± 1.1 mg/l as $CaCO_3$)

Mean measured copper conc. (µg/l)		Mean hatch (%)	1-30 Days		31-60 Days		
			Survival (%)	Mean total length (mm)	Survival (%)	Mean total length (mm)	Mean total wet wt.(mg)
24	A	98	1	16 (0.8)	0	-	-
	B	86	5[a]	16 (0.8)[a]	0[a]	-	-
18	A	92	26	17 (0.8)	61	26 (3.2)	180
	B	90	20[a]	17 (2.1)[a]	39	29 (4.7)[a]	230
12	A	76	36	21 (2.2)	69	34 (4.3)	360
	B	71	52	19 (2.3)	65	32 (4.3)	290
7	A	100	65	21 (2.5)	88	33 (4.7)	330
	B	80	52	21 (2.4)	92	33 (3.6)	350
6	A	80	49	19 (1.6)	47	31 (4.5)	300
	B	78	32	21 (2.5)	84	33 (5.7)	340
3	A	55	77	20 (2.1)	96	33 (4.5)	310
	B	67	50	21 (2.2)	96	32 (4.6)	280
Control	A	84	49	21 (2.3)	66	35 (4.1)	340
	B	64	59	22 (2.8)	86	35 (4.5)	400

[a] Denotes values significantly lower than the controls (Duncan's Multiple Range P=0.05)

TABLE 29 - MEAN PERCENTAGE HATCH OF EGGS, MEAN SURVIVAL, TOTAL LENGTHS AND WET WEIGHT OF CHANNEL CATFISH (Ictalurus punctatus) FRY CONTINUOUSLY EXPOSED TO COPPER IN HARD WATER (186.3 ± 38.7 mg/l as $CaCO_3$)

Mean measured copper conc. (µg/l)		Mean hatch (%)	1-30 Days		31-60 Days		
			Survival (%)	Mean total length (mm)	Survival (%)	Mean total length (mm)	Mean total wet wt. (mg)
66	A	24	0	-	-	-	-
	B	23	0[a]	-	-	-	-
34	A	35	0	-	-	-	-
	B	47	0[a]	-	-	-	-
19	A	40	14	21 (3.3)	80	25 (3.4)	170
	B	28	9[a]	21 (3.3)	80[a]	25 (3.4)[a]	170[a]
13	A	51	54	24 (2.5)	100	29 (4.6)	270
	B	29	48	24 (3.4)	95	30 (5.0)	320
10	A	46	53	27 (3.1)	100	30 (4.9)	280
	B	50	64	24 (2.7)	98	27 (4.4)	230
7	A	52	34	23 (2.7)	98	34 (4.2)	340
	B	72	72	23 (1.9)	96	33 (4.9)	340
Control	A	83	69	23 (1.9)	100	32 (4.2)	320
	B	46	68	24 (2.0)	100	34 (3.7)	370

[a] Denotes values significantly lower than the controls (Duncan's Multiple Range P=0.05)

Walleye (soft and hard waters)

As mentioned previously, exposures of walleye eggs and fry were generally unsuccessful due to difficulty in early feeding of fry. The following observations were made during the early stages of the copper exposures. In soft water 35 mg/l as $CaCO_3$), no walleye eggs hatched successfully during exposure to 91 µg/l copper and hatchability was significantly reduced by exposure to 47 µg/l (Table 30). In hard water (189 mg/l as $CaCO_3$) hatchability of walleye was similar for controls and eggs exposed to copper concentrations as high as 71 µg/l (Table 31). Percentage hatch for eggs in all experimental units ranged from 44-69%. In soft water, none of the walleye fry survived 30 days exposure to 47 or 21 µg/l copper, although survival was generally poor even in controls. In hard water, all walleyes including controls died very early in the exposure. Based on the limited available evidence, the MATC for copper and walleye is estimated to be between 13 and 21 µg/l in soft water, and greater than 71 µg/l in hard water.

TABLE 30 - MEAN PERCENTAGE HATCH OF EGGS, MEAN SURVIVAL AND TOTAL LENGTHS OF WALLEYE (*Stizostedion vitreum*) FRY CONTINUOUSLY EXPOSED TO COPPER IN SOFT WATER (35.0 ± 1.8 mg/l as $CaCO_3$)

Mean measured copper conc. (µg/l)		Mean hatch (%)	1-30 Days	
			Survival (%)	Mean total length (mm)
91	A	0	-	-
	B	0[a]	-	-
47	A	24	0	-
	B	10[a]	0	-
21	A	57	0	-
	B	56	0	-
13	A	53	12	10
	B	54	0	-
8	A	62	38	11
	B	71	16	12
3	A	53	24	9
	B	44	20	11
Control	A	46	18	11
	B	60	18	9

[a] Denotes values significantly lower than the controls (Duncan's Multiple Range P=0.05)

TABLE 31 - MEAN PERCENTAGE HATCH OF EGGS, MEAN SURVIVAL, TOTAL LENGTHS AND WET WEIGHT OF WALLEYE (Stizostedion vitreum) FRY CONTINUOUSLY EXPOSED TO COPPER IN HARD WATER (189.0 ± 33.0 mg/l as $CaCO_3$)

Mean measured copper conc. (µg/l)		Mean hatch (%)	1-30 Days Survival (%)
71	A	52	0
	B	44	0
38	A	59	0
	B	63	0
24	A	69	0
	B	61	0
17	A	56	0
	B	45	0
14	A	62	0
	B	61	0
9	A	58	0
	B	68	0
Control	A	61	0
	B	54	0

EXPOSURE OF FISH TO CADMIUM

Brook trout (soft water)

Hatchability of brook trout eggs in soft water (37 mg/l as $CaCO_3$) was similar for controls and eggs exposed to cadmium concentrations as high as 47 µg/l (Table 32). Survival of brook trout fry through 30 days post-hatch exposure was also not affected by exposure to cadmium concentrations as high as 47 µg/l. Total lengths of brook trout were significantly reduced by 30 days exposure to cadmium concentrations $\geq$ 10 µg/l.

Survival of brook trout was significantly reduced during 60 days exposure to test concentrations of cadmium $\geq$ 6 µg/l. Total length of brook trout exposed to these same concentrations was also significantly lower than that of control fish at this time. Total wet weight of brook trout after 60 days exposure to concentrations $\geq$ 3 µg/l cadmium was significantly less than controls.

TABLE 32 - MEAN PERCENTAGE HATCH OF EGGS, MEAN SURVIVAL, TOTAL LENGTHS AND WET WEIGHT OF BROOK TROUT (Salvelinus fontinalis) EGGS AND FRY CONTINUOUSLY EXPOSED TO VARIOUS CONCENTRATIONS OF CADMIUM IN SOFT WATER (37.0 ± 7.2 mg/l as $CaCO_3$)

Mean measured cadmium conc. (µg/l)		Mean hatch (%)	1-30 Days		31-60 Days		
			Survival (%)	Mean total length (mm)	Survival (%)	Mean total length (mm)	Mean total wet wt.(mg)
47	A	61	96	17 (2.9)^a	2^a	25 (3.8)^a	115^a
	B	70	89	17 (1.7)^a	2^a	27 (1.4)^a	136^a
24	A	74	72	19 (1.7)^a	4^a	25 (1.4)^a	135^a
	B	74	96	19 (1.9)^a	22^a	24 (3.1)^a	146^a
10	A	16	48	18 (1.5)^a	4^a	25 (1.4)^a	135^a
	B	74	100	18 (1.7)^a	38^a	24 (3.0)^a	123^a
6	A	65	99	20 (1.5)	50^a	25 (3.8)^a	143^a
	B	76	96	20 (1.5)	30^a	25 (2.5)^a	148^a
3	A	58	99	21 (1.6)	82	27 (3.3)	191
	B	73	100	22 (1.7)	78	28 (3.0)	183
1	A	78	100	22 (1.8)	90	29 (3.7)	232
	B	75	99	22 (1.5)	58	30 (3.1)	226
Control	A	75	100	22 (1.7)	100	29 (3.8)	240
	B	71	100	22 (1.4)	100	30 (3.8)	236

[a] Denotes values significantly lower than the controls (Duncan's Multiple Range P=0.05)

Based on this exposure, the MATC of cadmium for brook trout eggs and fry exposed in soft water is estimated to be between 1 and 3 µg/l.

Brook trout (hard water)

Hatchability of brook trout eggs in hard water (188 mg/l as $CaCO_3$) was generally lower than hatchability in soft water but indicated no significant effects resulted from exposure to cadmium concentrations as high as 91 µg/l (Table 33). Percentage survival of brook trout fry during 30 days post-hatch exposure was excellent and also indicated no effects of exposure to the cadmium concentrations tested. Total lengths of brook trout fry after 30 days exposure to 91, 50 and 21 µg/l cadmium were significantly lower than total lengths of control fish.

None of the brook trout fry survived 60 days exposure to 91 and 50 µg/l and survival was significantly reduced by 60 days exposure to 21 and 12 µg/l indicating an extremely cumulative effect during the 30-60 day exposure period. In addition, total length and total weight of surviving brook trout exposed to 21 and 12 µg/l cadmium were also reduced when compared with controls.

Based on these data, the MATC of cadmium for brook trout exposed in hard water (188 mg/l as $CaCO_3$) is estimated to be between 7 and 12 µg/l.

Channel catfish (soft water)

Exposure to cadmium concentrations as high as 54 µg/l had no significant effect on the percentage of channel catfish eggs which hatched successfully (Table 34). The survival of channel catfish fry was significantly reduced by 30 days exposure to cadmium concentrations $\geq$ 17 µg/l when compared with controls. Total lengths of catfish fry after 30 days were slightly less than that of controls in all cadmium concentrations but differences were not statistically significant.

During the 31-60 days post-hatch exposure, the survival of catfish fry which had survived the initial 30 day exposure was significantly reduced by exposure to 54 and 32 µg/l cadmium. However, survival was not significantly reduced by exposure to cadmium concentrations of 20 and 17 µg/l, which did significantly reduce survival during the initial 30 day exposure. Total lengths and wet weights of catfish fry after 60 days exposure to cadmium were highly variable and

TABLE 33 - MEAN PERCENTAGE HATCH OF EGGS, MEAN SURVIVAL, TOTAL LENGTHS AND WET WEIGHT OF BROOK TROUT (*Salvelinus fontinalis*) FRY CONTINUOUSLY EXPOSED TO CADMIUM IN HARD WATER (188.0 ± 27.0 mg/l as $CaCO_3$)

Mean measured cadmium conc. (µg/l)		Mean hatch (%)	1-30 Days		31-60 Days		
			Survival (%)	Mean total length (mm)	Survival (%)	Mean total length (mm)	Mean total wet wt.(mg)
91	A	51	100	16 (1.7)	0	-	-
	B	36	96	15 (1.7)[a]	0[a]	-	-
50	A	53	100	17 (1.5)	0	-	-
	B	54	100	17 (1.9)[a]	0[a]	-	-
21	A	48	100	18 (1.9)	14	21 (1.9)	85
	B	45	100	18 (1.4)[a]	4[a]	21 (2.3)[a]	85[a]
12	A	42	100	19 (1.5)	40	22 (2.3)	95
	B	56	100	19 (1.4)	50[a]	23 (1.9)[a]	84[a]
7	A	46	100	20 (1.4)	84	24 (1.6)	109
	B	49	100	20 (1.6)	78	24 (2.4)	115
3	A	46	100	21 (1.5)	78	24 (2.5)	114
	B	43	98	20 (1.5)	90	24 (2.6)	120
Control	A	50	100	21 (1.4)	88	25 (2.0)	125
	B	58	100	21 (1.0)	74	25 (2.0)	136

[a] Denotes values significantly lower than the controls (Duncan's Multiple Range P=0.05)

TABLE 34 - MEAN PERCENTAGE HATCH OF EGGS, MEAN SURVIVAL, TOTAL LENGTHS AND WET WEIGHT OF CHANNEL CATFISH (*Ictalurus punctatus*) FRY CONTINUOUSLY EXPOSED TO CADMIUM IN SOFT WATER (37.0 ± 1.3 mg/l as $CaCO_3$)

Mean measured cadmium conc. (μg/l)		Mean hatch (%)	1-30 Days		31-60 Days		
			Survival (%)	Mean total length (mm)	Survival (%)	Mean total length (mm)	Mean total wet wt.(mg)
54	A	93	0	-	0	-	-
	B	82	1[a]	19	0[a]	-	-
32	A	97	2	17 (1.2)	0	-	-
	B	98	2[a]	17 (1.2)	50[a]	24 (5.7)[a]	170
20	A	90	11	19 (1.9)	52	32 (6.4)	300
	B	86	4[a]	19 (1.9)	100	35 (5.0)	400
17	A	79	17	17 (1.2)	58	25 (5.1)	170
	B	51	30[a]	19 (1.9)	96	29 (3.4)	270
11	A	75	46	19 (1.5)	92	31 (2.6)	240
	B	55	40	19 (1.7)	85	30 (4.7)	240
6	A	63	60	20 (1.7)	86	31 (3.8)	270
	B	63	61	20 (1.8)	98	36 (3.7)	380
Control	A	77	63	21 (2.6)	84	35 (4.7)	380
	B	90	30	22 (3.3)	69	36 (5.2)	380

[a] Denotes values significantly lower than the controls (Duncan's Multiple Range P=0.05)

differences could not be shown to be statistically significant.

Based on these data, the MATC for cadmium and channel catfish exposed in soft water is estimated to be between 11 and 17 μg/l.

Channel catfish (hard water)

The percentage of channel catfish eggs which hatched successfully in hard water (185 mg/l as $CaCO_3$) was not significantly affected by exposure to cadmium concentrations as high as 59 μg/l (Table 35). After 30 days post-hatch exposure, survival of channel catfish was highly variable and no treatment related effects could be statistically verified. Total length of channel catfish after 30 days exposure to 59 μg/l cadmium was significantly reduced when compared with controls.

After 60 days post-hatch exposure, survival of channel catfish was excellent in all concentrations of cadmium which were tested and in controls. Total length and total wet weight of catfish exposed to 59, 33 and 17 μg/l cadmium for 60 days was reduced when compared with controls, indicating cumulative growth effects at these concentrations in hard water. Nearly identical concentrations of cadmium in soft water had affected survival of catfish after only 30 days exposure indicating that increased water hardness delayed or reduced the toxic effects of cadmium at these concentrations.

Based on these data, the MATC of cadmium for channel catfish eggs and fry exposed in hard water (185 mg/l as $CaCO_3$) is estimated to be between 12 and 17 μg/l.

Walleye(soft and hard waters)

Exposure to concentrations of cadmium as high as 55.0 μg/l in soft water (35.0 mg/l as $CaCO_3$) (Table 36), and as high as 86.7 μg/l in hard water (187.0 mg/l as $CaCO_3$) (Table 37) did not significantly affect the percentage of walleye eggs which hatched successfully. Difficulty in feeding newly hatched walleyes (previously discussed) resulted in extremely poor survival and the only significant observation was the complete mortality of walleye fry observed during the initial 10 days of exposure to 55 and 25 μg/l in soft water.

Based on these data, the MATC for walleye is estimated to be between 9 and 25 μg/l in soft water (35.0 mg/l as $CaCO_3$) and >86.7 μg/l in hard water (187.0 mg/l as $CaCO_3$).

TABLE 35 - MEAN PERCENTAGE HATCH OF EGGS, MEAN SURVIVAL, TOTAL LENGTHS AND WET WEIGHT OF CHANNEL CATFISH (Ictalurus punctatus) FRY CONTINUOUSLY EXPOSED TO CADMIUM IN HARD WATER (185.0 ± 35.0 mg/l as $CaCO_3$)

Mean measured cadmium conc. (µg/l)		Mean hatch (%)	1-30 Days		31-60 Days		
			Survival (%)	Mean total length (mm)	Survival (%)	Mean total length (mm)	Mean total wet wt. (mg)
59	A	81	38	21 (2.6)[a]	88	29 (3.6)[a]	230[a]
	B	81	10	21 (2.6)[a]	100	31 (3.6)[a]	270[a]
33	A	71	46	24 (2.8)	92	30 (4.5)[a]	280[a]
	B	86	81	23 (2.0)	98	31 (3.9)[a]	250[a]
17	A	57	41	24 (2.3)	98	29 (4.9)[a]	300[a]
	B	90	60	23 (1.9)	100	29 (3.4)[a]	290[a]
12	A	75	22	23 (2.2)	100	34 (3.1)	360
	B	61	47	24 (2.2)	98	33 (3.5)	340
5	A	86	53	25 (2.6)	94	33 (4.6)	320
	B	83	58	24 (2.4)	96	34 (4.0)	330
2	A	41	59	26 (2.3)	96	34 (3.2)	380
	B	81	78	25 (2.5)	100	32 (3.1)	340
Control	A	56	72	24 (2.0)	98	34 (3.3)	350
	B	43	53	26 (2.2)	98	33 (3.1)	340

[a] Denotes values significantly lower than the controls (Duncan's Multiple Range P=0.05)

TABLE 36 - MEAN PERCENTAGE HATCH OF EGGS, MEAN SURVIVAL AND TOTAL LENGTHS OF WALLEYE (Stizostedion vitreum) FRY CONTINUOUSLY EXPOSED TO CADMIUM IN SOFT WATER (35.0 ± 1.2 mg/l as $CaCO_3$)

Mean measured cadmium conc. (µg/l)		Mean hatch (%)	1-30 Days	
			Survival (%)	Mean total length (mm)
55	A	64	0[a]	-
	B	65	0	-
24	A	68	0[a]	-
	B	57	0	-
9	A	63	8	10 (1.0)
	B	61	2	10 (0.9)
4	A	61	12	12 (1.3)
	B	58	10	10 (1.7)
2	A	59	10	12 (1.9)
	B	69	18	12 (3.2)
0.9	A	64	14	12 (2.4)
	B	71	8	9 (1.5)
Control	A	64	2	13 (2.3)
	B	65	16	12 (0.6)

[a] Denotes values significantly lower than the controls (Duncan's Multiple Range P=0.05)

TABLE 37 - MEAN PERCENTAGE HATCH OF EGGS AND SURVIVAL OF WALLEYE (*Stizostedion vitreum*) FRY CONTINUOUSLY EXPOSED TO CADMIUM IN HARD WATER (187.0 ± 36.0 mg/l as $CaCO_3$)

Mean measured cadmium conc. (µg/l)		Mean Hatch (%)	1-30 Days Survival (%)
86.7	A	60	0
	B	52	0
44.3	A	48	0
	B	66	0
19.0	A	46	0
	B	61	0
8.4	A	48	0
	B	54	0
3.4	A	48	0
	B	48	0
1.3	A	49	0
	B	57	0
Control	A	37	0
	B	56	0

SECTION VI

DISCUSSION

The criteria utilized to estimate the maximum acceptable toxicant concentrations (MATC's) for metals tested in this study were generally survival and/or growth (determined by measurements of length and weight) of fry. In all cases, these parameters were affected at metal concentrations significantly lower than those which were observed to affect egg hatching. These data support the growing body of evidence that the eggs of fishes are generally more resistant to inimical chemicals than are other life stages.

Analysis of the MATC values generated from the results of this study indicated that measured concentrations of copper and cadmium which were effective in significantly reducing survival and/or growth of fish fry were similar and were generally an order of magnitude lower than those derived for lead and chromium with the same fish species. Lead ranked third in relative toxicity and chromium was the least toxic of the four metals tested, producing comparable effects only at concentrations 10-100 times greater than those for copper and cadmium (Table 38 and 39).

MATC's estimated for copper and cadmium from tests conducted in soft (35.0 - 37.5 mg/l as $CaCO_3$) and hard (185.0 - 189.0 mg/l as $CaCO_3$) water indicated that there were no significant differences related to water hardness for brook trout and channel catfish. In the case of walleyes, however, an increase in the hardness of exposure water apparently resulted in a significant increase in the estimated MATC's for both copper and cadmium.

A superficial analysis of the MATC's estimated for fish exposed to lead and chromium might indicate a wide range in sensitivity among the seven fish species tested. However, if the actual duration of exposure is considered (Table 40), it appears that the sensitivities of these species are not significantly different from one another. That is, the species with the lowest MATC's are those which were exposed for the longest period of time. Therefore, the actual duration of exposure should be considered in interpretation of data generated from chronic assays.

EXPOSURE OF FISH TO LEAD

While there are now many data in the literature describing the acute toxicity, generally under static test conditions, of metals to freshwater fish (e.g., see reviews by McKim _et_ _al_.,

TABLE 38 - SUMMARY OF THE MAXIMUM ACCEPTABLE TOXICANT CONCENTRATION (MATC) OF LEAD AND CHROMIUM FOR SELECTED FRESHWATER FISH SPECIES IN SOFT WATER (32.6 - 40.7 mg/l as $CaCO_3$)

Fish Species	MATC (µg/l)	
	Lead	Chromium
Rainbow trout (*Salmo gairdneri*)	>71<146	>51<105
Lake trout (*Salvelinus namaycush*)	>48<83	>105<194
Channel catfish (*Ictalurus punctatus*)	>75<136	>150<305
Bluegill (*Lepomis macrochirus*)	>70<120	>522<1122
White sucker (*Catostomus commersoni*)	>119<253	>290<538
Northern pike (*Esox lucius*)	>253<483	>538<963
Walleye (*Stizostedion vitreum*)	>237<397	>2167

TABLE 39 - SUMMARY OF THE MAXIMUM ACCEPTABLE TOXICANT CONCENTRATION (MATC) OF COPPER AND CADMIUM FOR SELECTED FRESHWATER FISH SPECIES IN SOFT (35.0 - 37.5 mg/l as $CaCO_3$) AND HARD (185.0 - 189.0 mg/l as $CaCO_3$) WATER

Fish Species	Water hardness	MATC (μg/l)	
		Copper	Cadmium
Brook trout (Salvelinus fontinalis)	soft	>3<5	>1<3
	hard	>5<8	>7<12
Channel catfish (Ictalurus punctatus)	soft	>12<18	>11<17
	hard	>13<19	>12<17
Walleye (Stizostedion vitreum)	soft	>13<21	>9<25
	hard	>71	>87

TABLE 40 - SUMMARY OF THE ACTUAL DURATION OF EXPOSURE TO LEAD AND CHROMIUM FOR SELECTED FRESHWATER FISH SPECIES

Fish Species	Duration of Exposure (days)
Rainbow trout (Salmo gairdneri)	95 - 97
Lake trout (Salvelinus namaycush)	111 - 115
Channel catfish (Ictalurus punctatus)	66 - 68
Bluegill (Lepomis macrochirus)	62
White sucker (Catostomus commersoni)	70 - 73
Northern pike (Esox lucius)	24
Walleye (Stizostedion vitreum)	39 - 42

1973, 1974; Leland et al., 1975), there are still relatively few data describing sublethal effects of this metal on the growth and development of early life stages of fish. Davies and Everhart (1973) report the results of a study in which the acute and chronic toxicity of lead [as lead nitrate, $Pb(NO_3)_2$] to rainbow trout in soft and hard water was determined. Data regarding hatchability of rainbow trout eggs exposed to lead concentrations ranging from 5.3 to 101.8 µg/l in soft water (28.1-28.7 mg/l as $CaCO_3$) were inconclusive since 100% egg mortality occurred in one replicate of controls and in at least one replicate of all lead treatments except at 50.9 µg/l. In our study (soft water, 34.6 mg/l as $CaCO_3$), hatchability was excellent (≥82%) in controls and all lead treatments to 443 µg/l. However, at 672 µg/l lead, only 28 and 29% of the exposed eggs hatched successfully.

Davies and Everhart observed significant differences in growth, determined as mean total length, among fish at the various lead treatments but concluded that these differences were not lead related but were due to the use of eggs of significantly different sizes, with the possibility of significant genetic differences in brood stock, to initiate this study. We observed significant differences in mean total lengths of fry exposed to lead concentrations ≥250 µg/l as compared with controls and those at lower treatments after 30 days of continuous exposure. Survival to 60 days post-hatch was near zero at all concentrations ≥250 µg/l lead and was severely reduced at 146 µg/l lead. Davies and Everhart similarly observed severely reduced survival (33%) after 6 months of exposure to 95.2 µg/l lead. Growth measurements for fry surviving 60 days exposure at all lead treatments were not significantly different from those of controls.

Physical abnormalities such as "black tail", scoliosis and caudal fin erosion were observed during the Davies and Everhart study. Black tail was the most common abnormality observed and its incidence was 42%, 91.3% and 100% for fish exposed to lead concentrations of 23.8, 47.6 and 95.2 µg/l, respectively. Based on black tail, Davies and Everhart estimated an MATC of >6.0 <11.9 µg/l for rainbow trout exposed to lead in soft water. Scoliosis in fry was also observed in our study but to a lesser degree. Rainbow trout fry exposed to 443 and 672 µg/l lead for 30 days post-hatch had a scoliosis incidence of 12 and 28%, respectively. Black tails were never observed on our fish. Based on our data, the MATC for rainbow trout exposed to lead is estimated to be >71 <146 µg/l. Discrepancies between our results and those of Davies and Everhart may be due to a number of factors. Nevertheless, these approximations of MATC's are comparable considering factors such as the shorter duration of our study, differences in fish populations and others.

Similar concentrations of lead, as determined for rainbow trout, were effective in significantly decreasing survival and growth of fry of lake trout, channel catfish and bluegill but among these, only bluegill were observed to be affected by scoliosis. White sucker, northern pike and walleye appeared to be more resistant to lead exposure and MATC's estimated for these species were 2-3 times higher than the others. Scoliosis was a very common and severe problem with white sucker exposed to the higher lead concentrations (253 and 483 µg/l).

Northern pike were difficult to study due to their cannibalistic behavior. Walleye were also difficult to feed adequately under these conditions. Continuous exposure to a lead concentration of 397 µg/l was associated with a high incidence of scoliosis not observed in controls or other lead treatments.

Based on the MATC values estimated for 7 species of freshwater fish chronically exposed to lead in soft water, the recommendation of a maximum lead in water concentration of 0.03 mg/l (National Academy of Sciences, 1973) appears to be adequate for the protection of most fishes in the aquatic environment.

EXPOSURE OF FISH TO CHROMIUM

There is a paucity of data regarding the effect of chromium on freshwater fish as a result of chronic exposure. Olson (1958) and Olson and Foster (1956, 1957), as reported in National Academy of Sciences (1973) and in McKim and Benoit (1971), exposed eggs and fry of rainbow trout and chinook salmon to chromium as sodium dichromate and determined that fry were more sensitive than eggs to chromium and that the effect of this metal was cumulative. Pickering (1971, unpublished data, in National Academy of Sciences, 1973) reported a safe concentration of 1.0 mg/l for chromium and fathead minnows in hard water. Benoit (1976, personal communication) determined that the MATC's for both rainbow and brook trout chronically exposed to chromium were between 0.2 and 0.4 mg/l. Based on these data, the maximum permissible concentration of chromium in the aquatic environment is recommended to be 0.05 mg/l (National Academy of Sciences, 1973).

A review of the MATC values generated in the present study indicate that safe concentrations vary from 51 to >2167 µg/l for the 7 freshwater fish species tested.

Chromium concentrations $\leq$194 µg/l were apparently safe over the 60 day exposure for the survival and growth in total length of rainbow trout but wet weights of fry were significantly lower than those of controls at concentrations ≥105 µg/l.

The survival and total length of lake trout exposed to concentrations as high as 822 µg/l for 60 days were apparently unaffected.

However, as with rainbow trout, deleterious effects on wet weight of fry were observed at chromium concentrations ≥194 µg/l.

Channel catfish survival and mean total length were significantly affected by the end of 30 days of exposure to chromium concentrations ≥305 µg/l. However, at the end of 60 days of exposure to 305 µg/l, survival, total length and wet weight of fry were no longer significantly different from controls. Possibly this was due to the hardiness of the fish which survived the initial 30 day exposure. Therefore, the MATC estimated for channel catfish is based on survival and total length data from the first 30 days of exposure.

There was great variability, some of which was due to feeding problems, between replicates during the exposure of bluegill to chromium. Variabilities were such that statistical significance could not be ascribed to most observations. However, wet weights of fry after 60 days exposure to 1122 µg/l chromium was significantly different from controls and was the criterion for estimation of the MATC.

The MATC for white sucker exposed to chromium in soft water was estimated on the basis of wet weight measurements of fry, since weights again seemed to be the most sensitive of the parameters monitored.

Generally, estimates of MATC values on the basis of northern pike and walleye data are questionable. Severe feeding problems with both of these species greatly influenced observations and precluded adequate statistical treatment of the data.

Despite the feeding problems experienced with northern pike and walleye, the majority of the data generated from these chromium exposures indicated a very significant cumulative effect on fry. This cumulative effect was especially obvious in the results of exposure of rainbow and lake trout to this metal. In each of these cases, initial exposures at mg/l concentrations resulted in significant inimical effects at the end of 60 days at all chromium concentrations to which fry were exposed. Each test was repeated with fry exposed to a lower range of concentrations and again, in each case, significant effects of chromium on fry were not detected until the end of 60 days of exposure.

From the data presented, it appears that the current recommendation is not stringent enough and the maximum permissible concentration of chromium in the aquatic environment should be reduced. Consideration of the magnitude of this reduction should obviously include an analysis of the chronic effects of chromium on aquatic invertebrates such as crustaceans as well as recognition of the cumulative toxicity of this metal.

EXPOSURE OF FISH TO COPPER

Many data considering the chronic toxicity of copper to freshwater fish are currently available. Mount (1968) reported that the concentration of copper (as $CuSO_4$) in hard water (200 mg/l as $CaCO_3$) which did not affect growth and reproduction of fathead minnows was between 3 and 8% of the 96 hour median tolerance limit, that is, between 15 and 33 μg/l, for that species. Similar tests with fathead minnows exposed to copper in soft water (31.4 mg/l as $CaCO_3$) indicated the MATC was between 10.6 and 18.4 μg/l (Mount and Stephan, 1969). Among the parameters measured and found to be affected by exposure to 18.4 μg/l copper were survival and length through 30-120 days continuous exposure. McKim and Benoit (1971) exposed brook trout to Cu(II) in soft water (45 mg/l as $CaCO_3$) for 22 months and from the results estimated a MATC between 9.5 and 17.4 μg/l. They found no significant difference in sensitivity between fry from parents previously exposed to copper and those from unexposed parents. A copper concentration of 32.5 μg/l significantly affected the hatchability of trout eggs but lower test concentrations did not. The growth of fry was reduced by all copper concentrations tested during the first 23 weeks of exposure. However, after 23 weeks, fish at concentrations of ≤ 9.5 μg/l grew as well as controls. These limits for the MATC were apparently confirmed for brook trout in a subsequent study by McKim and Benoit (1974) in which they attempted to determine whether or not shorter duration exposures of fish would yield comparable and meaningful data relative to their earlier study. Results of the latter study indicated that at copper concentrations ≤ 9.4 μg/l there were no effects on survival, growth, number of eggs produced, hatchability and survival of fry subsequently.

Drummond et al., 1973 attempted to identify parameters which would be useful and reliable in predicting long-term effects of copper on brook trout based on short-term exposures. Copper concentrations selected for this study were based on the effect-no effect concentrations reported by McKim and Benoit (1971) and parameters observed included cough frequency, locomotion activity and feeding behavior. Based on their results, Drummond et al. concluded that selected parameters were significantly affected at copper concentrations ≥ 6 µg/l, with greatest distinctions obvious at concentrations ≥ 9 µg/l.

Studies in which blood characteristics, such as red blood cell count, hematocrit, hemoglobin and associated enzymes, of freshwater fish exposed to copper were measured in an attempt to detect effect due to this exposure yielded results comparable to those from studies in which reproduction or other parameters were measured.

McKim et al., (1970) determined an MATC between 9.5 and 17.4 µg/l copper based on blood plasma glutamic oxalacetic transaminase (PGOT) concentrations for brook trout exposed for 6 to 21 days to cupric sulfate in soft water (46 mg/l as $CaCO_3$). Christensen et al. (1972) exposed brown bullheads to copper (as $CuSO_4 \cdot 5H_2O$) for 6, 30 and 600 days in hard water (202 mg/l as $CaCO_3$) and estimated an MATC between 11 and 16 µg/l.

The MATC values estimated for brook trout and channel catfish exposed to copper agree very closely with those previously reported. Brook trout egg hatchability was significantly reduced by exposure to concentrations ≥ 13 µg/l copper and total length and wet weight were reduced after 60 days exposure to 5 µg/l in soft water (37.5 mg/l as $CaCO_3$). Hard water (187.0 mg/l as $CaCO_3$) appeared to prolong the time to observed effect while not materially affecting the MATC value. That is, in hard water, effects appeared later in the exposure (31-60 days), but at similar concentrations (i.e., 8 vs. 5 µg/l). Channel catfish data followed a pattern similar to that of brook trout but at slightly higher concentrations, resulting in a higher range defining the estimated MATC. Survival of newly-hatched fry through the first 30 days of exposure was comparable in terms of both effective concentration and relative percentages in soft and hard water. MATC's estimated for channel catfish exposed to copper in soft and hard water are virtually identical.

Interpretation of data from walleye exposures is difficult due to significant problems in feeding the fry. For example, test concentrations of copper ≥ 47 µg/l significantly reduced hatching success of walleye eggs exposed in soft water while roughly comparable concentrations in hard water had no effect on hatchability of eggs, however, no fry (including controls) survived to day 30 in hard water while concentrations ≤8 μg/l copper in soft water had no significant effect.

In any event, current water quality criteria (National Academy of Sciences, 1973) recommend that the concentration of copper in the aquatic environment not exceed 0.1 of the 96-hour LC50 for the species of interest in any particular receiving water. Based on the data in this study, the application factor should probably be changed.

EXPOSURE OF FISH TO CADMIUM

Cadmium has been shown to be an extremely potent and cumulative toxicant. Pickering and Gast (1972) reported an estimated MATC between 37 and 57 μg/l cadmium for fathead minnows exposed to cadmium sulfate in hard water (200 mg/l as $CaCO_3$). Cadmium concentrations ≥57 μg/l significantly decreased survival of developing embryos, apparently the most sensitive stage in the life cycle of this fish. Concentrations ≤37 μg/l cadmium did not affect survival, growth or reproduction of these fish. Hatchability of fathead minnow eggs exposed to 57 μg/l cadmium was not significantly different from that of controls. These investigators observed a slow, cumulative mortality occurring during exposures.

Eaton (1974) estimated on MATC between 31 and 80 μg/l cadmium for bluegill chronically exposed to cadmium sulfate in hard water (200 mg/l as $CaCO_3$) under conditions replicating those of Pickering and Gast (1972). As in this earlier study, Eaton observed that fish mortalities generally occurred late into the exposure periods (i.e., after 141 days), thus corroborating the cumulative effects of cadmium on freshwater fishes.

In the present study, we observed that exposure to cadmium concentrations as high as 91 μg/l had no significant effect on hatchability of brook trout, channel catfish and walleye eggs in soft (35.0-37.0 mg/l as $CaCO_3$) and hard (185.0-187.0 mg/l as $CaCO_3$) water. Effects of cadmium on survival of brook trout fry were not manifested at any concentration during the first 30 days exposure in both soft (37.0 mg/l as $CaCO_3$) and hard (188.0 mg/l as $CaCO_3$) water. However, after 60 days, survival in soft water (37.0 mg/l as $CaCO_3$) was affected at cadmium concentrations of ≥6 μg/l. Similarly, at 60 days, these concentrations significantly affected total length and wet weight of brook trout fry. Wet weight of fry was also significantly reduced after 60 days exposure to 3 µg/l cadmium. In hard water (188.0 mg/l as $CaCO_3$), significant effects on brook trout fry survival, length and weight were observed in cadmium concentrations $\geq$12 µg/l after 60 days. Fry exposed to 7 µg/l of cadmium were not apparently affected.

The survival of channel catfish fry exposed to cadmium concentrations $\geq$17 µg/l for 30 days in soft water (37.0 mg/l as $CaCO_3$), was significantly reduced as compared to that of controls. The survival of catfish fry in hard water (185.0 mg/l as $CaCO_3$) was too variable for statistical treatment. After 60 days exposure to cadmium in soft water (37.0 mg/l as $CaCO_3$), significant effects on survival, length and weight were difficult to demonstrate due to the variability among replicates. In hard water (185.0 mg/l as $CaCO_3$), significant effects of cadmium on the latter two parameters could be established for concentrations $\geq$17 µg/l.

Therefore, MATC's for cadmium and channel catfish eggs and fry were estimated based on percentage survival at the end of 30 days exposure in soft water (37.0 mg/l as $CaCO_3$) and on total length and wet weight after 60 days exposure in hard water (185.0 mg/l as $CaCO_3$).

Previously mentioned difficulties in the feeding of walleye fry resulted in our inability to ascribe statistical significance to most of these data. However, exposure to cadmium concentrations $\geq$24 µg/l resulted in 100% mortality of walleye fry within 30 days exposure in soft water (35.0 mg/l as $CaCO_3$). There was some survival (2-18%) of fry in control and cadmium treatments $\leq$9 µg/l. In hard water (187.0 mg/l as $CaCO_3$) there was 100% mortality of all fry in control and all cadmium treatments.

No physical abnormalities were observed in any of the fish exposed to cadmium. Pickering and Gast (1972) reported observing many blood clots in the vascular system of cadmium-exposed fathead minnows. Sangalang and O'Halloran (1972,1973) observed damage to the testes of brook trout exposed to 25 µg/l cadmium (as cadmium chloride), for 24 hours and to 10 µg/l cadmium for 21 days in soft water (20.0 mg/l as $CaCO_3$). Gross damage included general discoloration ("dark purple-brown patches") of the testes. A histological examination of tissues revealed distended blood vessels with collapsed walls in some places and local accumulations of erythrocytes. These investigators also suggested that steroid (androgen) synthesis was affected in fish with damaged testicular tissues. None of this testicular damage was observed after exposure of brook trout to cadmium concentrations of from 0-5 µg/l. These data indicate an MATC between 5 and 10 µg/l based on testicular damage. These values agree quite well with the results of the present study.

Based on these data, it appears that the recommendation for a maximum cadmium concentration of 0.004 mg/l in water with a hardness $\leq$100 mg/l as $CaCO_3$ (National Academy of Sciences, 1973) may just be barely adequate and possibly should be decreased further. This same maximum concentration should be extended to species extant in water with a hardness of $\geq$100 mg/l as $CaCO_3$ since the currently recommended limit of 0.03 mg/l (National Academy of Sciences, 1973) is clearly inadequate to protect aquatic life.

The one significant problem in protocol encountered during this study was the rearing of both northern pike and walleye fry under test conditions. Both of these species are highly active, voracious feeders which require a stenothermal medium for optimum development during specific portions of their life cycle. Studies of pond culture of northern pike (White, 1968) and relationship of water temperature to egg hatching (Walker, 1968) indicate that temperatures of 10°-12°C are more favorable for hatching than the 17°-18°C temperatures recommended in the egg and fry protocol. Furthermore, a gradual increase in water temperature from 10°-12°C to 14°-15°C (rather than to 17°-18°C) would have been adequate to induce feeding without encouraging the excessive cannibalism experienced. Walker (1968) recommended the stocking of tanks of swim-up muskellunge fry with white sucker fry to provide an adequate food source. In the present study, we found that during the first few weeks of exposure of northern pike fry, we could provide adequate numbers of white sucker fry as food. Northern pike fry appeared to feed most readily on forage fish which were 1/2 to 2/3 their own size. When offered fish food significantly smaller or larger than this range, northern pike fry became cannibalistic. Possibly, rearing these fry at lower temperatures (i.e. 14°-15°C) would help to minimize cannibalism.

For walleye, it has been suggested (Lloyd L. Smith, Jr., 1974, personal communications) that to maximize feeding, fry should be maintained in aquaria with illumination from above only. The use of this procedure apparently results in a more even distribution of fish in the aquarium and improved feeding activity. A gradual increase in water temperature from 15°C to 18-21°C during swim-up may also favor increased feeding.

In general, the recommendations of maximum permissible concentrations of 0.05 mg/l for chromium, 0.1 of the 96-hour LC50 for copper and 0.0004-0.004 mg/l in soft water and 0.0003-0.03 mg/l in hard water for cadmium in the aquatic environment (National

Academy of Sciences, 1973) appear to be inadequate based on the results of the present study. In order to suggest concentrations which may be more appropirate for the protection of the aquatic environment, a rationale which related acute and chronic toxicity, the bioconcentration potential of these metals and a margin of safety was employed. Acute toxicity data for freshwater fishes exposed to these metals were compiled and then compared with chronic toxicity data from this study to produce a factor relating the two. The factor calculated was 0.001. Multiplying the lowest concentrations reported for 96-hour LC50 values of each metal and appropriate freshwater fishes by this factor and then applying a safety factor resulted in the following recommendations of maximum permissible concentrations of each metal in the aquatic environment: 0.03 mg/l for chromium, 0.1 μg/l for copper and 0.01 μg/l for cadmium. These concentrations, based on the results of the present study, appear to be more appropriate than are others presently available.

In summary, the good correlation between MATC values estimated from the results of these relatively short-term exposures and those from much longer-term, full-chronic studies indicates that egg and fry studies are an cost-effective (in terms of both time and money) and reliable means of assessing the potential hazard to selected freshwater fish species of certain compounds which may impact the aquatic environment.

SECTION VII

LITERATURE CITED

APHA, AWWA and WPCF, 1971. *Standard Methods for the Examination of Water and Wastewater*, 13th edition. Amer. Public Health Assoc., Washington, DC: 874 pp.

Battelle-Columbus Laboratories, 1971. Effects of chemicals on aquatic life. Water Quality Criteria Data Book, Vol. 3. U.S. Environmental Protection Agency, Water Pollut. Control Res. Series No. 18050GWV05/71.

Brungs, W.A., 1969. Chronic toxicity of zinc to the fathead minnow, *Pimephales promelas* Rafinesque. Trans. Amer. Fish. Soc. 98: 272-279.

Brungs, W.A., E.N. Leonard and J.M. McKim, 1973. Acute and long-term accumulation of copper by the brown bullhead, *Ictalurus nebulosus*. J. Fish. Res. Bd. Can. 30: 583-586.

Cairns, J., Jr. and A. Scheier, 1957. The effects of temperature and hardness of water upon the toxicity of zinc to the common bluegill (*Lepomis macrochirus* Raf.) Notulae Natur. (Philadelphia) no. 299: 1-12.

Christensen, G.M., J.M. McKim, W.A. Brungs and E.P. Hunt, 1972. Changes in the blood of the brown bullhead (*Ictalurus nebulosus* Leseur) following short and long term exposure to copper (II). Toxicol. Appl. Pharmacol. 23: 417-427.

Davies, P.H. and W.H. Everhart, 1973. Effects of chemical variations in aquatic environments: Volume III Lead toxicity to rainbow trout and testing application factor concept. U.S. Environmental Protection Agency, Ecological Res. Series No. EPA-R3-73-011C: 80 pp.

Drummond, R.A., W.A. Spoor and G.F. Olson, 1973. Some short-term indicators of sublethal effects of copper on brook trout, *Salvelinus fontinalis*. J. Fish. Res. Bd. Can. 30: 698-701.

Eaton, J.G., 1970. Chronic malathion toxicity to the bluegill (*Lepomis macrochirus* Rafinesque). Water Res. 4: 673-684.

Eaton, J.G., 1974. Chronic cadmium toxicity to the bluegill (*Lepomis macrochirus* Rafinesque). Trans. Amer. Fish. Soc. 4: 729-735.

Erickson, S.J., 1972. Toxicity of copper to *Thallasiosira pseudonana* in unenriched inshore seawater. J. Phycol. 8: 318-323.

Hermanutz, R.O., L.H. Mueller and K.D. Kempfert, 1973. Captan toxicity to fathead minnows (*Pimephales promelas*), bluegills (*Lepomis macrochirus*) and brook trout (*Salvelinus fontinalis*). J. Fish. Res. Bd. Can. 30: 1811-1817.

Leland, H.V., E.D. Copenhaver and D.J. Wilkes, 1975. Heavy metals and other trace elements. J. Water Pollut. Control Fed. 47: 1635-1656.

Lemke, A.E., 1969. A water hardener for experimental use. J. Amer. Water Works Assoc. 61: 415-416.

Macek, K.J., M.E. Barrows, R.F. Krasny, and B.H. Sleight, 1975. Bioconcentration of 14C-pesticides by bluegill sunfish during continuous aqueous exposure. Proc. of Symposium on Structure Activity Correlation in Studies of Toxicity and Bioconcentration with Aquatic Organisms. Canada Centre for Inland Waters, Burlington, Ont.

Macek, K.J., K.S. Buxton, S.K. Derr, J.W. Dean and S. Sauter, 1976. Chronic toxicity of lindane to selected aquatic invertebrates and fishes. U.S. Environmental Protection Agency, Ecological Res. Series No. EPA-600/3-76-046: 55 pp.

Macek, K.J., K.S. Buxton, S. Sauter, S. Gnilka, and J.W. Dean, 1976. Chronic toxicity of atrazine to selected aquatic invertebrates and fishes. U.S. Environmental Protection Agency, Ecological Res. Series No. EPA-600/3-76-047: 55 pp.

McKee, J.E. and H.W. Wolf, eds., 1963. Water quality criteria. 2nd edit. State Water Quality Control Board, Sacramento, Calif: 548 pp.

McKim, J.M. and D.A. Benoit, 1971. Effects of long-term exposures to copper on survival, growth, and reproduction of brook trout (*Salvelinus fontinalis*). J. Fish. Res. Bd. Can. 28: 655-662.

McKim, J.M. and D.A. Benoit, 1974. Duration of toxicity tests for establishing "no effect" concentrations for copper with brook trout (*Salvelinus fontinalis*). J. Fish. Res. Bd. Can. 31: 449-452.

McKim, J.M., G.M. Christensen and E.P. Hunt, 1970. Changes in the blood of brook trout (*Salvelinus fontinalis*) after short-term and long-term exposure to copper. J. Fish Res. Bd. Can. 27: 1883-1889.

McKim, J.M., G.M. Christensen, J.H. Tucker, D.A. Benoit and M.J. Lewis, 1973. Effects of pollution on freshwater fish. J. Water Pollut. Control Fed. 45: 1370-1407.

McKim, J.M., G.M. Christensen, J.H. Tucker, D.A. Benoit and M.J. Lewis, 1974. Effects of pollution on freshwater fish. J. Water Pollut. Control Fed. 46: 1540-1591.

Mount, D.I., 1966. The effect of total hardness and pH on acute toxicity of zinc to fish. Air Water Pollut. 10: 49-56.

Mount, D.I., 1968. Chronic toxicity of copper to fathead minnows (*Pimephales promelas*, Rafinesque). Water Res. 2: 215-223.

Mount, D.I. and W.A. Brungs, 1967. A simplified dosing apparatus for fish toxicology studies. Water Res. 1: 21-29.

Mount, D.I. and C.E. Stephan, 1967. A method for establishing acceptable toxicant limits for fish-malathion and the butoxyethanol ester of 2,4-D. Trans. Amer. Fish. Soc. 96: 185-193.

Mount, D.I. and C.E. Stephan, 1969. Chronic toxicity of copper to fathead minnow (*Pimephales promelas*) in soft water. J. Fish. Res. Bd. Can. 26: 2449-2457.

National Academy of Sciences, 1973. Water Quality Criteria, 1972. U.S. Environmental Protection Agency, Ecological Res. Series No. EPA-R3-73-033: 594 pp.

Olson, P.A., 1958. Comparative toxicity of Cr (VI) and Cr (III) in salmon. Hanford biology research-annual report for 1957 (HW 53500) Hanford Atomic Products Operation, Richland, WA: 215-218 (see National Academy of Sciences, 1973).

Olson, P.A. and R.F. Foster, 1956. Effect of chronic exposure to sodium dichromate on young chinook salmon and rainbow trout. Hanford biology research-annual report for 1955 (HW 41500) Hanford Atomic Products Operation, Richland, WA: 35-47 (see National Academy of Sciences, 1973).

Olson, P.A. and R. F. Foster, 1957. Further studies on the effect of sodium dichromate on juvenile chinook salmon. Hanford biology research-annual report for 1956 (HW 47500) Hanford Atomic Products Operation, Richland, WA: 214-224 (see National Academy of Sciences, 1973).

Pagenkopf, G.K., R.C. Russo and R.V. Thurston, 1974. Effects of complexation on toxicity of copper to fishes. J. Fish. Res. Bd. Can. 31: 462-465.

Perkin-Elmer, 1973. Analytical Methods for Atomic Absorption Spectrophotometry. Perkin-Elmer Corp., Norwalk, Connecticut.

Pickering, Q.H. and M.H. Gast, 1972. Acute and chronic toxicity of cadmium to the fathead minnow (*Pimephales promelas*). J. Fish. Res. Bd. Can. 29: 1099-1106.

Pickering, Q.H. and C. Henderson, 1965. The acute toxicity of some heavy metals to different species of warm water fishes. Proc. 19th Indust. Waste Conf., Purdue Univ.: 578-591.

Sangalang, G.B. and M.J. O'Halloran, 1972. Cadmium-induced testicular injury and alterations of androgen synthesis in brook trout. Nature 240: 470-471.

Sangalang, G.B. and M.J. O'Halloran, 1973. Adverse effects of cadmium on brook trout testis and on *in vitro* testicular androgen synthesis. Biol. Reprod. 9: 394-403.

Steele, R.G.D. and J.H. Torrie, 1960. *Principles and Procedures of Statistics*. McGraw-Hill, New York: 481 pp.

Tabata, K., 1969. Studies on the toxicity of heavy metals to aquatic animals and the factors to decrease the toxicity. IV. On the relationship between the toxicity of heavy metals and the quality of environmental water. Bull. Tokai Fish. Res. Lab. 58: 243-253

U.S. EPA, 1971. Methods for chemical analysis of water and wastes. Analytical Quality Control Laboratory, Cincinnati, OH., U.S. Environmental Protection Agency, Water Pollut. Control Res. Series No. 1805GWV05/71.

U.S. EPA, 1972a. Proposed recommended bioassay procedure for egg and fry stages of freshwater fish. U.S. Environmental Protection Agency, National Water Quality Laboratory, Duluth, MN.

U.S. EPA, 1972b. Handbook for analytical quality control in water and wastewater laboratories, Analytical Quality Control Laboratory, Cincinnati, OH.

Walker, K.W., 1968. Temperature control in northern pike and muskellunge egg hatching. p 13-17. In: Proc. of North Central Warm Water Fish Culture Workshop, Feb. 15-16, Ames, IO.

White, B., 1968. Northern pike pond culture. p 21-23. In: Proc. of North Central Warm Water Fish Culture Workshop, Feb. 15-16, Ames, IO.